PB86-166014

Microbial Activity in Composting Municipal Sewage Sludge

Univ. of Cincinnati, OH

Prepared for

(U.S.) Environmental Protection Agency
Cincinnati, OH

Feb 86

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-166014

EPA/600/2-86/025
February 1986

MICROBIAL ACTIVITY IN COMPOSTING MUNICIPAL SEWAGE SLUDGE

by

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CR-807852-01-0

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CINCINNATI, OHIO 45268

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/2-86/025	2.	3. RECIPIENT'S ACCESSION NO. PB86 166014/AS
4. TITLE AND SUBTITLE Microbial Activity in Composting Municipal Sewage Sludge		5. REPORT DATE February 1986
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J. Robie Vestal and Vicky L. McKinley		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Cincinnati Cincinnati, Ohio 45221		10. PROGRAM ELEMENT NO. CAZB1B
		11. CONTRACT/GRANT NO. CR 807852-01-0
12. SPONSORING AGENCY NAME AND ADDRESS Water Engineering Research Laboratory, Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final Project Report
		14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES

Project Officer: Atal E. Eralp (513) 684-2621

16. ABSTRACT

Research was conducted to identify the most important operational parameters which limit the growth and decomposition activity of composting sludge microbiota. Sensitive and nonselective biochemical methods of monitoring microbial biomass and activity were tested and used to study the interactions between the microbial communities and temperature, the primary factor affecting their activity during composting. Optimum temperatures for microbial activity and biomass were generally within the 35° to 55° range. Biokinetic analyses revealed that compost samples from low-temperature (25° to 45°C) areas of the pile had much greater microbial activity [^{14}C] substrates than did samples from high temperature (60° to 75°C) areas. The microbial communities became better adapted to increasing temperatures as composting progressed, but their temperature optimum was never greater than 55°C. Biomass was monitored by measuring the lipid phosphate content (an important cell membrane component) of the compost. Other parameters that were measured included the moisture content, total organic content, total protein content, and pH.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 130
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxics Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

The report concerns composting as a sewage sludge treatment technology. Composting of municipal sludges is of interest as both a method of disposal and resource recovery which avoids many of the pollution problems arising from traditional methods of waste treatment. This report details research aimed as elucidating the role of microorganisms in the composting process, in an effort to improve the efficiency of municipal sewage sludge composting.

Francis T. Mayo, Director
Water Engineering Research Laboratory

PREFACE

In a world of limited resources and ever-increasing population and industrialization, the importance of resource recovery and of the environmental impacts of pollution necessitate the use of rational and efficient methods for the recycling and disposal of all wastes and byproducts. Municipal wastes and sewage sludges are some of the largest sources of both valuable organic nutrients and hazardous wastes. The composting process can be a useful method of recycling some of these wastes and avoiding the environmental pollution caused by their disposal. If the composting process is to reach its maximum potential in this regard, however, an acceptance of the fact that it is primarily a biological process, and not a chemical or physical one, is essential, as is an understanding of the properties of the microorganisms involved and the biological principles that govern their lives.

ABSTRACT

Research was conducted to identify the most important operational parameters which limit the growth and decomposition activity of composting sludge microbiota. Sensitive and nonselective biochemical methods of monitoring microbial biomass and activity were tested and used to study the interactions between the microbial communities and temperature, the primary factor affecting their activity during composting. Optimum temperatures for microbial activity and biomass were generally within the 35° to 55°C range. Biokinetic analyses revealed that compost samples from low-temperature (25° to 45°C) areas of the pile had much greater microbial activity (measured as the rate of incorporation or mineralization of [^{14}C] substrates) than did samples from high-temperature (60° to 75°C) areas. The microbial communities became better adapted to increasing temperatures as composting progressed, but their temperature optimum was never greater than 55°C. Biomass was monitored by measuring the lipid phosphate content (an important cell membrane component) of the compost. Other parameters that were measured included the moisture content, total organic content, total protein content, and pH.

This report was submitted in fulfillment of Contract No. CR-807852-01-0 by the Department of Biological Sciences, University of Cincinnati, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period of January 30, 1981, to August 30, 1984.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

ATP --adenosine triphosphate
^{o}C --degrees Celsius (centigrade)
CPM --counts per minute
DPM --disintegrations per minute
g --grams
h --hours
kg --kilograms
M --Molar concentration
min --minutes
ml --milliliters
N --Normal concentration
nm --nanometers
ns --not significant (statistically)
TCA --trichloroacetic acid

SYMBOLS

^{14}C --isotopic carbon
CO_2 --carbon dioxide
HgCl --mercuric chloride
ln --natural logarithm
uCi --microcurie
ug --microgram
n --sample size for the statistic
p --p value of statistical significance
% --percent
PO_4 --phosphate
r --Pearson's correlation coefficient
U --uniformly isotopically labeled

ACKNOWLEDGMENTS

We thank Dr. Atal E. Eralp, Water Engineering Research Laboratory, U.S. Environmental Protection Agency, for his interest, encouragement, and numerous helpful discussions. We also appreciate the cooperation of Dr. Harry A.J. Hoitink and Dr. Geoff Kuter of the Ohio Agricultural Research and Development Center, Ohio State University, Wooster, Ohio, in coordinating our separate projects and the numerous helpful discussions that resulted. Special thanks go to Mr. Doug Entler, who coordinated the experimental composting trials and provided the automatically recorded temperature data. We greatly appreciate the cooperation of Paygro, Inc. (espescially Mr. Karl Kipp and Mr. Fred DeKamp) for providing the facilities and manpower for the experimental composting runs. Thanks are also extended to Mr. Richard Ryan of Compost Systems Co. for his help and cooperation throughout this project. Municipal sewage sludge was provided by R.A. Monteith, Bureau of Engineering, Akron, Ohio through an EPA agreement with H. Hoitink. Special thanks go to C.L.H. Barnhart, G.D. Brunner, C.A. Meininger, B.J. Moller, and D.J. Schlemm for their assistance with the field work.

SECTION 1

INTRODUCTION

Large aggregations of organic-rich matter have long been known to heat up and become increasingly humified over extended periods. These effects are brought about by the activity of the indigenous microbial community, which decomposes the usable matter for energy and growth substrates, producing metabolic heat as a by-product. In organic piles of sufficient size and insulation, this metabolic heat is trapped and can elevate the temperatures within the pile to in excess of 80°C within a few days (20,25,31,42,55). The production of composted material through this process has been a means of recycling organic waste products throughout much of history. Recently the process has become an important means of disposing of municipal solid waste and sewage sludge (20,25,26,42,43,58). The primary goals of composting in solid waste management are to rapidly reduce the pathogens, odors, putrescible organic matter, moisture, and bulk, and to produce a biologically stabilized material (25,43,55).

No general agreement has yet been reached on the best conditions and procedures for optimizing the efficiency of decomposition (stabilization) during the composting process, so a wide variety of methods are currently practiced (25). However, temperature is generally agreed to be the most critical parameter influencing the rate of composting and the quality of the product, given reasonable initial environmental conditions of moisture, free air space, pH and nutrients (25,43,45,46,54), and provided that oxygen does not become generally limiting.

Many researchers have largely ignored the vital role that microorganisms play in this process, or they have made erroneous assumptions concerning their physiological and biochemical properties. One such assumption is that as temperatures in a composting pile become very hot (greater than 60° to 65°C), the facultatively and extremely thermophilic microorganisms will become very active and numerous as the mesophilic organisms decline, leading to increased rates of composting. However, these types of rapid succession and adaptation to such extremely high temperatures are not generally found in groups of heterotrophic microorganisms in nature (2,10). Some composting practices are predicated on the idea that such high temperatures are necessary to insure both rapid pasteurization and stabilization (20,43,45,46,49), even though a temperature of 55°C for at least 3 days has been found to be adequate for very significant reduction of pathogens in aerated static pile systems (17,24). Others have proposed that the accumulation of heat is the critically limiting factor in microbial decomposition, and that aeration systems can be used to dissipate some of the excess heat in order to optimize the rate of stabilization (26,27,38,42).

Many of the findings on temperature optimization during composting have been in conflict (25,42). These discrepancies may be partly due to the indirect and incomplete nature of many of the studies concerning microbial activity and biomass in composting material. Direct comparisons between different temperature regimes are thus impossible. Estimates of microbial decomposition in composting material have typically been made using such indirect indices as the overall temperature of the composting pile, odors (38), moisture content (27,38,54), carbon dioxide evolution from the composting pile (54), or oxygen uptake by the composting pile (49). In most cases, concurrent assessments of microbial biomass were not made, or they were aimed only at quantifying the numbers of surviving pathogens (20) or other specific groups using selective isolation techniques (25).

The purpose of this study was to provide a better understanding of the factors influencing the microbial activities in composting sewage sludge. The activity and biomass of the microbial community as a whole were accurately and consistantly measured to permit conclusions about an optimal temperature range for rapid decomposition during composting to be made. Basic information leading to a better understanding of rapid thermogenic microbial successions was also obtained.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The major conclusions of this study include the following:

1. The optimum temperature range for composting sewage sludge in a forced-aeration batch, static-pile system appears to be 35° or 45° to 55°C. The lower limits of this range are much less distinct than the upper limits. These conclusions are based on measurements of the levels of microbial activity (rates of [^{14}C]substrate incorporation or mineralization) and biomass (lipid phosphate concentration). Microbial activity and biomass dropped off very rapidly as composting temperatures exceeded 55^{14}C. Other indirect and much less responsive indicators of microbial activity and biomass (such as the compost pH and protein concentrations) were also optimal within this temperature range. The lowest levels of microbial activity were always found in compost samples at very high temperatures (> 60°C).

2. As composting progressed, evidence showed that the microbiota were adapting to higher temperatures, but no microbial communities acclimated to temperatures above 55°C. No evidence that extremely thermophilic organisms (having temperature optima >60°C) played a measurable role in composting was found.

3. Compared with piles composted simulataneously at 60° to 85°C, piles aerated to maintain temperatures at or below 55°C showed significant improvements in the rates of microbial metabolism and growth.

The recommendations that naturally follow from these conclusions include the following:

1. Composting should be done at temperatures within the stated optimum range whenever possible.

2. Nonselective, responsive, and sensitive methods for analyzing microbial activity and biomass should be used to monitor the process when necessary.

3. Further studies should be done to determine optimum ranges for other composting parameters (moisture, free air space, particle size, oxidation-reduction potential, pH, and carbon-to-nitrogen ratios) with regard to microbial decomposition.

SECTION 3

MATERIALS AND METHODS

COMPOSTING FACILITIES

Composting was performed in a large production-scale composting bin operated by Paygro, Inc., 11000 Huntington Rd., South Charleston, Ohio 45368. The composting piles measured approximately 6 by 24 by 3 m. Air was forced up through the piles from the perforated base of the composting bin. These facilities have been described in detail previously (30,42,48,59).

EXPERIMENTAL COMPOSTING RUNS

The experiments reported here were performed during experimental composting trials managed by H.A.J. Hoitink and G.A. Kuter of the Ohio Agricultural Research and Development Center, Ohio State Univ. (30), or by the Akron Composting Demonstration Project, Burgess and Niple, Ltd., 46 South Summit St., Akron, Ohio (Appendix III of ref 30), who where also cooperating with the U.S. Environmental Protection Agency. Appendix I provides the starting and ending dates of each of these trials and cross-references the composting "run" numbers used in this report with the composting "trial" numbers used by the other investigators.

Temperatures within the composting piles were continuously monitored at 80 different locations by Dr. Hoitink's group using thermocouples (type K; Marlin Manufacturing Corp., Cleveland, Ohio) which were connected to a microprocessor (model RP-1D; Kaye Instruments, Bedford, Mass.). These temperature data were used to automatically regulate the operation of the aeration fans so as to keep the mean composting pile temperature below a certain preselected level. This temperature feedback regulation system operates in a manner similar to those previously described for other facilities (26,38). The mean reading of these 80 temperature sensors is reported as the mean compost pile temperature. This may not be the true mean temperature of the pile, due to the limitations associated with the locations of the probes within the temperature gradients (30), but it should accurately reflect the overall relative changes in temperature which occurred. The entire composting facility is housed under a roof, alleviating any problems associated with precipitation.

COMPOSTING MATERIAL

Primary raw sewage sludge (undigested and dewatered) from either Columbus, Ohio or Akron, Ohio was mixed with bulking agents (hardwood bark chips and sawdust, mostly oak) and previously-composted sewage sludge. The volumetric ratios of these components varied during runs 1 through 4 (30). During runs 5 and 6 a ratio of 1.0 sludge to 1.06 bark and sawdust to 1.46 recycled compost was used in order to generate enough recycled material for subsequent runs (Appendix III of ref. 30). During runs 7 through 9 "steady state" conditions of operation were achieved using a ratio of 1.0 sludge to 0.39 bark and sawdust to 1.21 recycled compost (Appendix III of ref. 30). For run 10 the ratio used was 1.0 sludge to 0.4 bark to 0.3 sawdust to 2.8 recycled compost, yielding 44.2% dry solids in the starting material (30). Cow manure was used instead of sewage sludge in two of the early runs (Appendix 1). Approximately 295,000 kg (wet weight) of the initial mix was composted during each batch processing run. The entire composting pile was removed from the bin and then replaced (or "turned") usually once or twice during each run, at about weekly intervals, to provide more free air space and a more even exposure of the material to elevated temperatures and aeration.

SAMPLING PROCEEDURES

Sampling and microbiological assays were performed on several different days during each composting run. On each sampling day material was taken from several locations within the composting pile. In this composting system a temperature gradient usually exists from the upper central portion of the pile (the hottest) to areas near the walls and floor of the composting bin (the coolest)(30), making it possible to sample from a variety of temperature areas on any particular day. An attempt was made to sample from areas similar in other respects (moisture and aeration) during runs 8 through 10, so most samples were taken from a depth of not more than 20 to 40 cm below the upper surface of the pile. Immediately prior to removing each sample from the pile, the temperature was determined using a mercury thermometer. The microbiological assays were begun immediately after sampling in a laboratory trailer adjacent to the composting facility.

Composting runs 1 through 6 were essentially preliminary experiments conducted to determine which methods of sampling and microbiological analysis would work best in this system to give the greatest efficiency and the least variability. Thus, the methods of analysis vary considerably throughout these early runs, and therefore the data obtained in different runs may not be directly comparable. However, the results obtained from runs 7 through 10 were obtained in a much more consistant manner, and these experiments were designed to test specific hypotheses.

MICROBIAL BIOMASS DETERMINATIONS

An accurate, non-selective measure of viable microbial biomass was needed for this experimental approach. The quantification of ATP (adenosine triphosphate) has been shown in many instances to be a good indicator of total viable microbial biomass (37), since ATP is found in all living cells and is generally destroyed by ATPase enzymes very rapidly after the death of the cell. However, in sediments and soils it has often been found that the many multivalent ions and fulvic acids present interfere with the commonly used luciferin-luciferase assay for ATP (14,18,36,37). In addition, it has been found that microbes under conditions of stress or physiological change have variable cellular ATP pools (37,39), and thus ATP may not be a good biomass indicator to use when responses of microbial activity to potentially stressful conditions are being studied.

Another biochemical method of microbial biomass determination that is much less prone to such interferences is the analysis of extractible lipid phosphate. All cells have phospholipids, primarily in their cell membranes, although the amounts per cell are greater in the eubacteria than in eukaryotic organisms (i.e. fungi). Phospholipids are not found in storage lipids, and do not fluctuate widely with physiological status. Although phospholipids do not turn over quite as rapidly as ATP does, they generally do not appear to persist long enough after cell death to preclude the detection of biomass changes within the microbial community over the kinds of time periods dealt with in our sampling schemes (62). Lipid phosphate is easily measured at levels of 10^{-9} moles (62), allowing for the detection of about 10^9 bacteria the size of _E. coli_ (63).

ATP Proceedures

Extraction of ATP-- Several methods of ATP extraction were compared for efficiency, consistancy and variability during runs 1 through 3. ATP was extracted from the compost immediately after sampling. The ATP was then either assayed immediately (during run 3), or was frozen until it could be assayed using a scintillation counter (runs 1 and 2). Additionally, experiments were conducted in the laboratory to compare various ATP extraction techniques on composted material.

To compare the efficiencies of various extraction methods, 0.5 g (wet weight) of compost was added to either 1) 5.0 ml of 0°C 0.6 N sulfuric acid plus NaEDTA (sodium ethenediamine tetraacetate) as a chelator (36), 2) 5.0 ml of 0°C 0.6 N sulfuric acid in 8 g/l oxalic acid (18), 3) 8 ml of boiling 0.01 M alkaline glycine-MgEDTA (pH 10; 56), or 4) 8 ml of boiling McIlvaine's buffer (0.04 M Na_2HPO_4, adjusted to pH 7.70 with 0.02 M citric acid; 14). In cold extractions the samples were vortexed for 1 min., while boiling extractions were carried out for 5 min.

Extracts were centrifuged for 10 min. at high speed in a table top centrifuge, and 3.5 ml of the cold extract or 1.0 ml of the hot extract supernatants were transfered to a plastic test tube, brought up to 10.0 ml with deionized distilled water, and frozen.

Analysis of ATP--

Once the ATP had been extracted from a sample, it was quantified by the luciferin-luciferase bioluminescence assay (37,39). Two different proceedures were used for the assay.

During runs 1 and 2, the amount of light produced was measured using a scintillation counter (Packard Tri-carb model 3003). One ml of sample extract was tranfered to a 7 ml plastic mini-scitillation vial, and 0.5 ml of firefly lantern extract (FLE-50, Sigma Chemical Co.) containing luciferin and luciferase was added. The sample was then vortexed for exactly 10 sec., and then loaded into the scintillation counter (set at 52% gain, dicriminators 30-1000; 1). Exactly 60 sec. after the addition of the FLE-50, counting of the sample was begun. Counts were recorded after 10 sec. of counting.

During run 3 the ATP assays were carried out at the composting site using a portable luminometer (Lumac M 1070). After 100 ul of sample extract was placed in a plastic test tube in the luminometer, 100 ul of purified luciferin-luciferase (Lumit, Lumac B.V., Schaesberg, The Netherlands) was added and a 10 sec. integrated count was obtained.

In both of the above proceedures, an internal standard was used to calibrate efficiency by adding 0.50 ml of a 1 ug/ml ATP standard solution to two of the samples. A standard curve in the range of 0.1 to 1.0 ug ATP was also obtained using this standard solution. Standard curves from the scintillation counter took the form of ln ATP concentration= (slope x ln counts) + intercept, and the curves from the luminometer were linear.

Lipid Phosphate Analysis

Total lipids were extracted in triplicate from 10 g (wet weight) of compost (runs 1 through 7) or from 15 ml of homogenized sample (runs 8 through 10), using chloroform and methanol (8). During runs 1 through 8 the compost was transported to the laboratory on ice and then extracted 4 to 5 h after sampling. During runs 9 and 10, however, the samples were extracted at the composting site 30 to 60 min. after sampling. All extractions and transfers were carried out in glass or teflon containers.

The extracted lipids were then digested by refluxing in 32% perchloric acid at 180°C for 2 h in a test tube without going to dryness (59,61). The resulting inorganic phosphate was then quantified colorimetrically using a Spectronic 20 (Bausch and

Lomb) at 830 nm after performing the molybdate blue reaction (19). The results are expressed as umoles pf phosphate per gram (dry weight) of compost (umol PO_4 g^{-1}).

PROTEIN ANALYSIS

Amounts of total protein can sometimes be used as an indirect indicator of both microbial biomass and decompositional changes in a mixture of substrates. Since proteins do not generally turn over (recycle) as rapidly as ATP or phospholipids, total proteins are not good indicators of viable microbial biomass, particularly in dynamic systems. However, total protein analyses were carried out on the samples from runs 9 and 10 to see if any changes in the chemical composition of the material during composting could be detected.

Several different methods of total protein analysis were initially compared for efficiency and variability for use with compost. After comparing several variations of the Biuret (16), Lowry (16,35), and Coomassie Blue (9,50,53) methods, it was found that the Lowry method (35) was the most sensitive and replicable for use with composting sewage sludge. In addition, the Lowry method gave the least variable response to equal quantites of several different purified proteins (bovine serum albumin (BSA), cellulase, peroxidase, B-glucosidase, and lysozyme).

Lowry Protein Proceedures

The Lowry (Folin-Ciocalteu; 35) method is very sensitive (1 ug) and very commonly used to assay total proteins in a variety of applications. However, it may give variable results for "total protein" content depending upon the particular amino acids making up the proteins present. Of the methods tested in this study, however, the Lowry gave the least variation in this regard. Proteins are assayed by an initial Biuret reaction followed by the reduction of phosphomolybdate-phosphotungstate salts by the tyrosine- and tryptophan-containing proteins.

For each assay, 0.5000 g of dried compost was extracted with 10 ml of 0.1 N NaOH for 3 days in a screw-top plastic centifuge tube, with daily vortexing. After centrifugation, the protein in 2 ml of supernatant was precipitated with 4 ml of 10% cold TCA (trichloroacetic acid) overnight. The precipitate was redissolved in 2 ml of 0.1 N NaOH for the assay.

Standard cuves were prepared using 1 mg/ml BSA. For each assay, 5 ml of freshly prepared alkaline copper reagent was added to 1.0 ml of sample extract. Fresh alkaline copper reagent was prepared by mixing, in order, 1 ml of 1% copper sulfate ($CuSO_4 . 5H_2O$), 1 ml of 2% sodium tartrate, and 98 ml of 2% alkaline sodium carbonate (2% Na_2CO_3 in 0.1 N NaOH). After 10 min. at room temperature, 0.25 ml of Folin reagent (Phenol reagent 2 N from Fisher Chemical Co.) was added with vortexing. The blue color was then read on a Spectronic 20 at 500 nm. Results are reported as % protein (w/w) of compost.

MICROBIAL ACTIVITY

The relevant determination of heterotrophic microbial activity during composting was the major aim of this project. As with the measurement of microbial biomass, methods which were as non-selective and accurate as possible were chosen. The measurement of rates of isotopically-labelled subsratate incorporation into microbial lipids has been found to be a useful indicatior of microbial activity in decomposing plant litter (24,39,40) and in sediments. $_{14}$Respiration rates can also be accurately measured using ^{14}C-substrates, by capturing and counting the $^{14}CO_2$ evolved.

Substrate Incorporation Rates

All substrate incorporation assays were performed a short time after sampling. Compost homogenates (2 ml) were incubated with 0.1 ml of [U-^{14}C]acetate (0.2022 to 0.2398 uCi, 0.293 to 0.338 ug), 0.1 ml of [U-^{14}C]glucose (0.2300 to 0.2500 uCi, 0.153 to 0.158 ug), or 0.1 ml of [U-^{14}C]glutamate (1.9110 to 2.2135 uCi, 0.0109 to 0.0126 ug) for 1 h at specific temperatures. In preliminary experiments it was determined that the incorporation of [^{14}C]acetate into microbial lipids in compost was linear ($r=0.9999$; $p < 0.01$) for at least 90 min., and began to taper off slightly after 120 min. ($r=0.9947$, $p < 0.01$). The rate of incorporation of the label into lipids was determined by extracting the total lipids and quantifying the radioactivity by liquid scintillation counting, as previously described (39). Abiotic controls recieved 0.5 ml of 0.0037 M HgCl12 15 min. before the addition of the labeled substrate.

The resulting data (dpms/h=disintegrations per minute per hour) were then adjusted for biomass (dpms per h per micromole of PO_4) or grams (dry weight) of compost (dpms per gram). Since batches of labeled substrate having slightly different activities were sometimes used, the data were also divided by the number of dpms of substrate added during at the beginning of the incubation, leaving a unitless numerator (decimal percent incorporation per hour per unit of biomass or weight).

Substrate Mineralization Rates

During runs 3 through 7, mineralization rates were determined using double vial radiorespirometry (41) at room temperature. However, these results often did not correlate well with substrate incorpoation rates which were obtained from samples incubated at the sampling temperature. Thus, during run 8 mineralization rates were determined using the serum-vial method (filter-paper wick CO_2

trap) described previously (23,39). These assays were performed very shortly after sampling, incubating at the sampling temperatures. Compost homogenates (5 ml) were incubated with 0.1 ml of L-[U-^{14}C]glutamate (0.1911 uCi, 0.0109 ug) in stoppered serum vials for various lengths of time. Abiotic controls recieved 1 ml of 0.0037 M $HgCl_2$ in 10% TCA 15 min. before the addition of the labeled substrate. The incubation was stopped and the carbon dioxide was trapped in the liquid by injecting 0.2 ml of 40% (wt/vol) KOH (potassium hydroxide) through the rubber stopper.

The vials were then transported back to the laboratory, the rubber stoppers were removed, and replaced with stoppers from which a plastic well was hanging. A piece of fluted filter paper (2 x 5 cm) soaked with 0.2 ml of phenethylamine (Fisher scintillation-grade) was placed in the well. An injection of 0.2 ml of 6 N H_2SO_4 then released the carbon dioxide from solution, to be trapped on the filter paper. After 2 h the filter paper was counted in 5 ml of scintillation cocktail {4% 2,5-diphenyloxazole, 0.2% 1,4-bis(2-[5-phenyloxazolyl])benzene in toluene}. This same cocktail was used for scintillation counting throughout this project, and all counts (cpms) were corrected to disintegrations (dpms) using the channels-ratio method. These data are presented in the same form as the incorporation data (per hour per micromole PO_4).

STATISTICAL ANALYSIS

Most of the results shown in this report are the mean (± one standard deviation) of three replicate analyses. Exceptions include determinations of dry weight (determined by heating 2.5 g samples of compost at 60°C to constant weight in quadruplicate) and total organic content (determined by ashing the compost from the dry weight analyses at 500°C for 2 h in quadruplicate). Single determinations of pH were made for each sample during runs 6 through 8 by mixing 5.0 g (wet weight) of compost with 10 ml of deionized distilled water using a glass stirring rod for 1 min., and then using a pH electrode (Fisher Scientific Co.) to measure the pH of the slurry. Triplicate determinations of pH were made during runs 9 and 10, and the geometric mean is reported.

Correlation coefficients (expressed as Pearson's r) of the replicate means were calculated using the "Pearson's Correlation" subprogram of SPSS (the Statistical Package for the Social Sciences). Significance levels reported are for two-tailed tests, except where noted. Microbial activity measures (substrate incorporation rates and substrate mineralization rates) were log transformed (using a natural logarithm, ln) prior to statistical analysis to improve the homogeneity of variance (homoscedasticity) of the data (52). Alternatively, an arcsine tranform was performed on the activity measures expressed per unit of biomass (PO_4) when they were correlated with lipid phosphate biomass, since this is a ratio correlation.

In all cases the number of samples used in determining a particular statistic is reported (n). In a few correlation analyses, the data from certain sampling days was excluded from the analysis (usually day 0 or day 1), since the data from these days are not representative of that found during the composting process, but rather before it, and thus tends to confound the results. In addition, day 19 was excluded from the run 9 analyses because the aeration fans had been turned on continuously since day 17 in order to dry the material further before removal, and therefore the temperatures were abnormally low for that time during the composting process.

Partial correlations were calculated using the "Partial Correlation" subprogram of SPSS, and Student's t-tests for differences between means were calculated using the "T-Test" subprogram of SPSS. Two-tailed tests of significance were performed except where noted.

SECTION 3

RESULTS

During composting runs 1, 2 and 3, various methods of sampling and analysis were tested in order to determine which methods would be relevant to answering the questions posed by this study. During runs 4 through 6, the methods which had been chosen were "fine tuned", and more extensive sampling was carried out. Experiments performed during runs 7 through 10 were designed to answer specific questions about the microbial community in composting sewage sludge.

COMPOSTING RUN #1

Samples were taken from 4 different depths from the surface of the pile on three different sampling days (Table 1). Biomass was estimated by measuring ATP content (boiling phosphate buffer extraction method) and by measuring the lipid phosphate content of the compost. Lipid phosphate biomass estimates appeared to vary inversely with temperature, but the correlation was not significant, probably due to the small sample size (Table 2). ATP biomass estimates had very large variances, so an experiment comparing various ATP extraction methods was performed (see methods section). Cold acid extractions were found to yield greater than 60 times more luminescent counts than the boiling buffer extractions. Others have also found cold sulfuric acid extractions of ATP to be more efficient in compost that bioling buffer extractions (34). The sulfuric acid-oxalate solution gave about 1.5 to 2 times more counts than the sulfuric acid-EDTA solution, so the acid-oxalate method was chosen for use in composting run #2.

COMPOSTING RUN #2

Duplicate samples were taken at two depths on three different sampling days (Table 3). Again, lipid phosphate biomass decreased with increasing sampling temperature, and moisture decreased with depth, but the statistics were not significant (Table 4).

Comparisons of biomass estimates made by measuring ATP or lipid phosphate indicated that lipid phosphate would be a more consistant and reliable estimate of biomass for use in compost. The coefficients of variance for ATP measurements were much larger and more variable (e.g. 30.1, 27.4, 32.6, 18.6) than those for lipid phosphate measurements (e.g. 6.77, 1.03, 9.93, 21.50) from

Table 1: Sampling temperature, moisture content, and lipid phosphate biomass of sewage sludge compost samples taken at various depths during composting run #1. Values shown for dry weight (n=4) and lipid phosphate (n=3) are a mean, standard deviation (S.D.), and coefficient of variance (C.V.).

Composting Run #1 Starting Date: 26 May 1981

Day	Depth (cm)	Temp. (°C)	Dry Wt. (%)	S.D.	C.V.	Lipid Ph. (µmol/g)	S.D.	C.V.
* 9	5	30	39.66	1.79	4.50	1.2489	0.2682	21.48
	25	60	41.28	0.45	1.08	0.9068	0.0614	6.77
	80	-	41.36	2.58	6.25	0.8081	0.0084	1.03
	132	-	42.76	1.48	3.46	0.8266	0.0821	9.93
** 15	5	29	50.34	1.73	3.43	1.2132	0.2290	18.89
	5	41	48.46	0.82	1.69	0.4334	0.0720	16.50
	80	40	58.11	1.43	2.46	0.6733	0.0313	4.64
	132	34	62.45	0.63	1.01	0.7252	0.0082	1.13
24	5	-	55.25	0.83	1.50	0.6702	0.1672	24.95
	25	-	67.70	0.49	0.72	0.6800	0.0744	10.94
	80	-	69.13	0.31	0.44	0.7827	0.0741	9.47
	132	-	69.74	0.30	0.42	0.7664	0.0212	2.77

* Compost pile was turned on day 4.

** Compost pile was turned on day 13.

Table 2: Matrix of correlation coefficients (r) for the parameters (Table 1) measured during composting run #1.

Correlation Matrix: Run #1

	Lipid Ph.	Dry Wt.	Depth	Time
+Temp.	-0.3508	-0.2928	0.2163	-0.4070
Time	-0.3954	0.8847 ###	0.0053	
Depth	0.2092	0.3345		
Dry Wt.	-0.4255			

$\#\ p < 0.05$

$\#\#\ p < 0.02$

$\#\#\#\ p < 0.01$

$n = 12$

$+n = 6$

Run #1, Day 15 and 24 only

Biomass	Dry Wt.	Depth
-0.9256	-0.0230	0.0391
-0.0893	0.6741	0.0483
-0.0332	0.6694	
-0.0334		

$n = 8$

$+n = 4$

Table 3: Sampling temperature, moisture content, and lipid phosphate biomass of sewage sludge compost samples taken at various depths during composting run #2. Values shown for dry weight (n=4) and lipid phosphate (n=3) are a mean, standard deviation (S.D.), and coefficient of variance (C.V.).

Composting Run #2   Starting Date: 24 June 1981

Day	Depth (cm)	Temp. (°C)	Dry Wt. (%)	S.D.	C.V.	Lipid Ph. (µmol/g)	S.D.	C.V.
0	5	23	45.87	0.39	0.85	2.5744	0.2754	10.70
	5	23	52.34	5.32	10.16	0.9916	0.2067	20.84
	25	35	47.06	0.72	1.52	1.8693	0.2199	11.76
	25	35	60.61	4.21	6.95	0.6264	0.0327	5.22
6	5	53	37.80	2.02	5.33	1.0806	0.1610	14.89
	5	53	45.53	0.58	1.28	1.0940	0.1060	9.72
	25	57	50.04	1.76	3.51	1.4480	0.1400	9.64
	25	47	50.45	1.07	2.13	1.1900	0.0570	4.82
* 13	5	47	49.67	0.90	1.82	1.1180	0.0640	5.76
	5	46	48.11	1.60	3.33	1.3930	0.0440	3.16
	25	-	58.76	0.16	1.97	1.2050	0.0240	1.98
	25	37	51.65	1.54	2.99	1.6490	0.6820	41.38

* Compost pile was turned on day 7.

Table 4: Matrix of correlation coefficients (r) for the parameters (Table 3) measured during composting run #2.

Correlation Matrix: Run #2

	Biomass	Dry Wt.	Depth	Time
+Temp.	-0.3671	-0.3434	0.0228	0.5357
Time	-0.1383	-0.5137	0.0000	
Depth	-0.0550	0.5733		
Dry Wt.	0.2028			

$* \ p < 0.05$

$** \ p < 0.02$

$*** \ p < 0.01$

$n = 12$

$+ \ n = 11$

Run #2, Days 6 and 13 only

	Biomass	Dry Wt.	Depth	Time
+Temp.	-0.5211	-0.4725	-0.2252	-0.7505 *
Time	0.3620	-0.2369	0.0000	
Depth	0.5285	0.6740		
Dry Wt.	0.3511			

$n = 8$

$+ \ n = 7$

the same compost samples. In addition, the two measurements of biomass did not correlate well with each other in this system. Since the ATP assay is known to be very sensitive to chemical interference, and ATP itself is very labile in the presence of certain chemical species, it was decided that the lipid phosphate method would be a better estimator of microbial biomass in the complex organic and inorganic milieu of composting sewage sludge.

COMPOSTING RUN #3

During runs 3 and 4, cow manure was composted instead of municipal sewage sludge. In addition, the piles were divided into two parts. In one section (the low-temperature pile), an attempt was made to keep the temperatures relatively low (mean < 50°C) by using increased aeration. The other section (the high-temperature pile) was allowed to get much hotter, since the set point for the aeration fans was higher. These different sections are refered to as the "Treatments (pile)" in the tables. Starting with this run, microbial activity was estimated, as well as micribial biomass. This was accomplished by measuring the rates if incorporation of [^{14}C]acetate, [^{14}C]glucose, and [^{14}C]glutamate into microbial lipids. These substrates were chosen as being representative of the types of simple substrates the organisms might encounter in composting sewage sludge. Acetate is a simple acid which can directly enter into fatty acid and lipid biosynthesis. Glucose is a simple sugar which can easily enter the energy-yielding catabolic pathways, as well as the biosysthetic pathways. Glutamate is a common amino acid, one of the building blocks of proteins, and can also enter the catabilic pathways. The rates of incorporation of each of these substrates into lipids is expressed in Table 5, both in terms of the rate per gram (dry weight) of compost, and as the rate per unit of biomass (umol of lipid phosphate). The former expression tends to combine the biomass and activity of the heterotrophic microbial community per unit of compost, while the latter expression corrects for biomass, giving the average metabolic activity of an individual microorganism.

The activity assays were all incubated at 36°C on day 1, while on day 7 the low-temperature pile samples were incubated at 60°C and the high-temperature pile samples were incubated at 70°C, since these were the means of the sampling temperatures for those groups of samples. The mean temperatures of the two different piles did not differ as much as had been anticipated, with the mean temperature of the low-temperature pile on day 7 being 60°C, and the mean temperature of the high-temperature pile being 63°C. This may explain why the t-tests to discern differences between the biomass or the activity of the samples from the two different piles did not show any significant differences, except that glutamate incorporation rates per unit of biomass were higher in the low-temperature pile samples on day 1.

However, when all of the data from run 3 was pooled for a

Table 5: Sampling temperature, moisture content, lipid phosphate biomass, and microbial activity (rates of C-14 labled acetate, glucose or glutamate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) of cow manure compost samples taken from various depths in either the low-temperature pile or the high-temperature pile during composting run #3. Values shown for dry weight (n=4), lipid phosphate (n=3), and microbial activity (n=3) are means, standard deviations (S.D.), and coefficients of variance (C.V.).

Composting Run #3 Starting Date: 13 August 1981

Day	Treatment (pile)	Depth (cm)	Temp. (°C)	Dry Wt. (%)*	Lipid Ph. (µmol/g)	S.D.	C.V.	Acetate (per g)	Glucose (per g)	Glute.+ (per g)	Acetate (per PO4)	Glucose (per PO4)	Glute.+ (per PO4)
0		5	29	53.39	3.678	0.324	8.81	7.6046	4.2841	2.8979	2.0676	1.1648	0.7879
		80	29	51.67	3.521	0.310	8.80	6.3224	3.5758	3.1057	1.7956	1.0156	0.8685
1	low	15	40	51.18	2.709	0.230	8.49	5.7730	2.1230	2.3470	2.1310	0.7838	0.8664
	low	80	33	51.73	2.513	0.196	7.81	4.2140	2.3490	2.1490	1.6770	0.9346	0.8552
	high	15	36	50.45	1.895	0.141	7.47	7.0240	1.9520	1.6020	3.7060	1.0300	0.8456
	high	80	34	50.68	2.739	0.737	27.00	1.6730	1.4510	1.5800	0.6109	0.5298	0.5767
7	low	15	66	51.59	1.938	0.050	2.55	0.4164	0.4456	1.4690	0.2148	0.2300	0.7580
	low	80	58	51.60	1.882	0.084	4.45	0.4545	0.2898	1.1510	0.2415	0.1540	0.6118
	high	15	70	51.80	2.119	0.048	2.29	0.3925	0.2953	1.3710	0.1852	0.1394	0.6472
	high	80	70	53.39	1.493	0.113	7.59	0.2714	0.2790	1.1050	0.1818	0.1868	0.7399

* C.V. < 6.00

+ Glute. = Glutamate

correlation analysis (Table 6), microbial biomass (lipid phosphate) and all microbial activity measures, except for glutamate incorpoation per unit of biomass, were highly significantly inversely correlated with temperature. This indicates that increasing temperature may have caused significant decreases in microbial biomass and activity. Figure 1 shows the decrease in biomass with increasing sampling temperature, while Figures 2-4 show the trends in microbial activity over temperature. Biomass and activity also appear to have declined significantly over the composting time, however, making it difficult to stictly seperate the effects of time and temperature in this case. Time and temperature were correlated also, indicating that sampling temperatures rose significantly over time. Thus, increased temperatures may indeed have caused the decline in microbial activity and biomass.

COMPOSTING RUN #4

This experimental run was similar to run 3 in that cow manure was composted, the pile was divided into low- and high-temperature sides, and similar measurements of microbial biomass and activity were made (Table 7). The mean temperatures of the two different sections, measured by means of the temperature probes, are shown in Table 8 for each sampling day. As in run 3, biomass and activity declined significantly over time. The negative correlations (Table 9) of biomass and activity with temperature were not significant in run 4, however, except for glucose incorporation ($p < 0.05$). Time and temperature were not significantly correlated in this run, and thus the negative correlations that are present with temperature are probably due primarily to the effect of temperature alone.

Good seperation of mean temperatures was achieved in the different piles during this run, however, (Table 8) which allowed for differences between the two piles to be observed. Lipid phosphate biomass was significantly higher in the low-temperature pile ($p=0.022$) when all the data were combined. Acetate incorporation per gram of compost was greater in the low-temperature pile on day 10 ($p=0.047$), and glutamate incorporation rates fell just below the level of significant difference. Again, the small sample sizes obtained during this run made it difficult to observe statistical differences between treatments.

In Figure 5 it can be seen that the maximum lipid phosphate levels were found in samples from the 40 to 45°C range. Figures 6-8 show the levels of microbial activity in relationship to sampling temperature. The maximum activity was found at about 50°C. The higher activities found above 55°C on day 15 compared with day 6 may be due to some microbial adaptation to higher temperatures over time.

Table 6: Matrix of correlation coefficients (r) for parameters (Table 5) measured during run #3.

Correlation Matrix: Run #3 ++

	Lipid Phosphate (µmol/g)	Glutamate Incorp. (per PO4)	Glucose Incorp. (per PO4)	Acetate Incorp. (per PO4)	Glutamate Incorp. (per g)	Glucose Incorp. (per g)	Acetate Incorp. (per g)	Dry Weight (%)	Depth (cm)	Treatment (pile temp.)	Time (days)
Temperature	-0.7708 ###	-0.4416	-0.9299 ###	-0.8235 ###	-0.7951 ###	-0.9375 ###	-0.9033 ###	0.2084	-0.0681	0.2970	0.9762 ###
Time	-0.7674 ##	-0.5211	-0.9524 ###	-0.8630 ###	-0.8227 ###	-0.9537 ###	-0.9356 ###	0.1917	0.0305	0.2091	
Treatment	-0.4610	-0.4166	-0.2968	-0.1910	-0.5535	-0.3830	-0.3096	-0.1091	0.0243		
Depth	-0.0770	-0.2523	-0.1528	-0.2832	-0.1604	-0.1182	-0.2314	0.0612			
Dry Wt.	0.1417	0.0453	-0.0842	-0.2264	0.0945	0.0083	-0.1251				
Acetate/g	0.7029 #	0.7094 #	0.9824 ###	0.9665 ###	0.8455 ###	0.9513 ###					
Glucose/g	0.8598 ###	0.6215	0.9707 ###	0.8485 ###	0.9318 ###						
Glutamate/g	0.9227 ###	0.6459 #	0.8460 ###	0.6948 ###							
Acetate/PO4 +	0.1657	0.7342 #	0.9386 ###								
Glucose/PO4 +	0.7488 ##	0.6960 #									
Glutamate/PO4 +	0.4020										

+ arcsine transformed for ratio correlations with lipid phosphate

++ using ln tranforms of microbial activity measures

$p < 0.01$

$p < 0.02$

# $p < 0.05$

n = 10

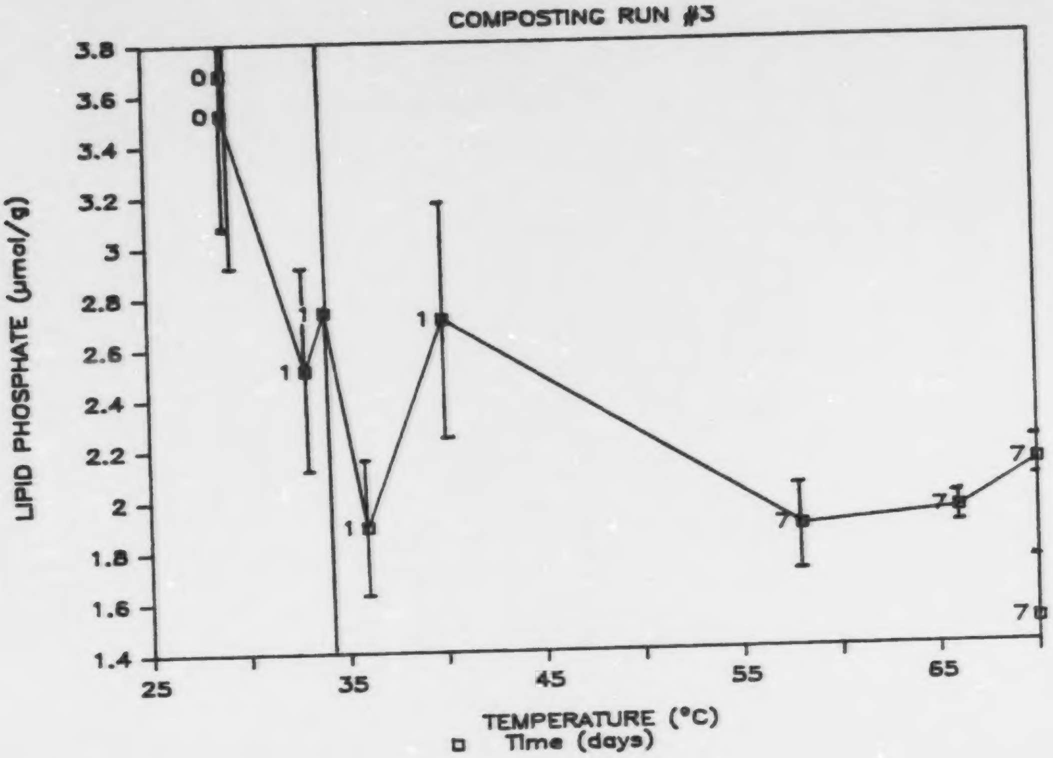

Figure 1. Microbial biomass, measured as the lipid phosphate concentration, in response to temperature in composting cow manure on various days (indicated by the numbers associated with the data points) after the beginning of composting run #3. Biomass values are shown as the mean ± one standard deviation (n=3).

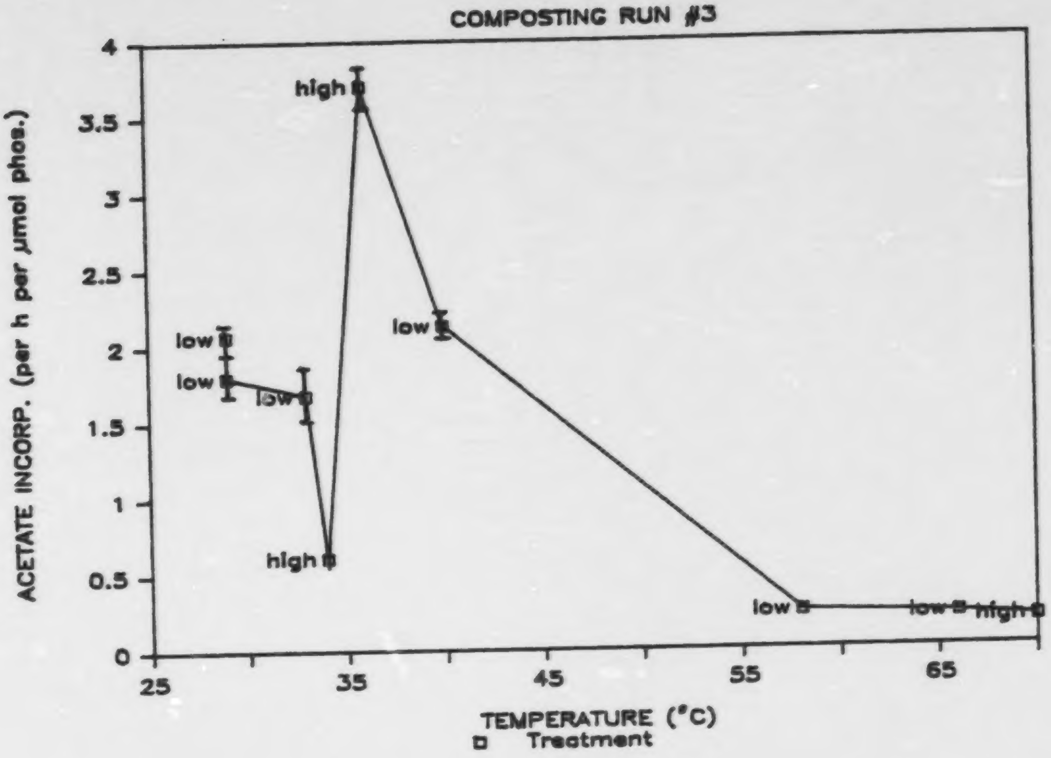

Figure 2. Microbial activity, measured as the rate of [^{14}C]acetate incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #3. Activity values shown are the mean ± one standard deviation (n=3).

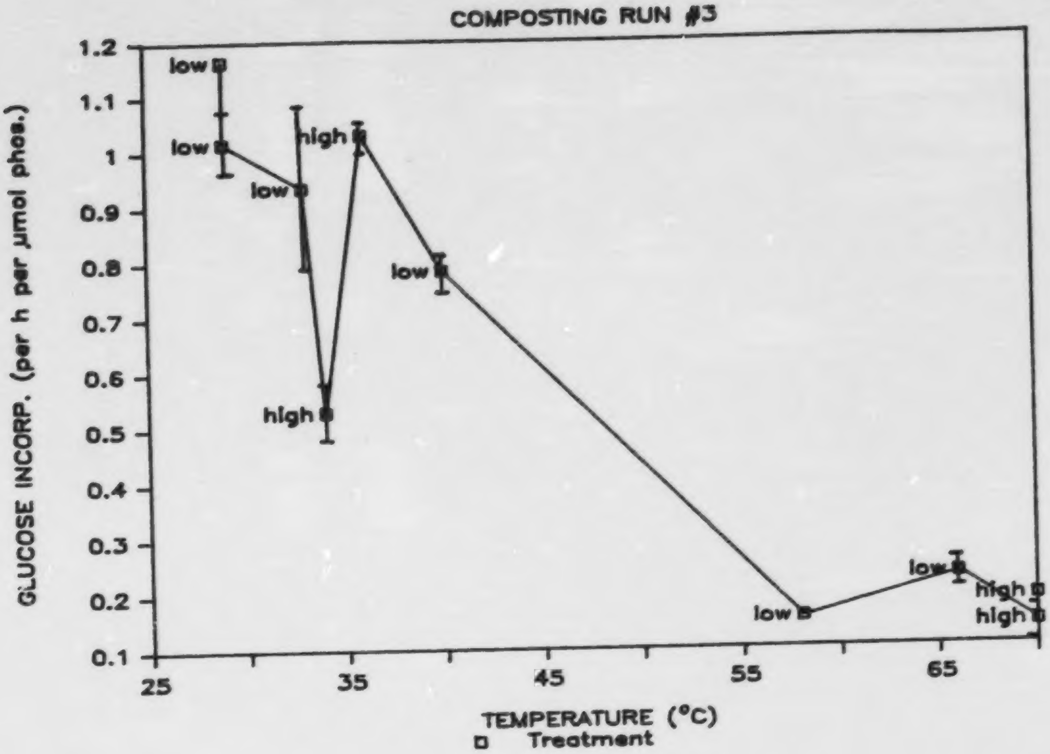

Figure 3. Microbial activity, measured as the rate of [^{14}C]glucose incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #3. Activity values shown are the mean ± one standard deviation (n=3).

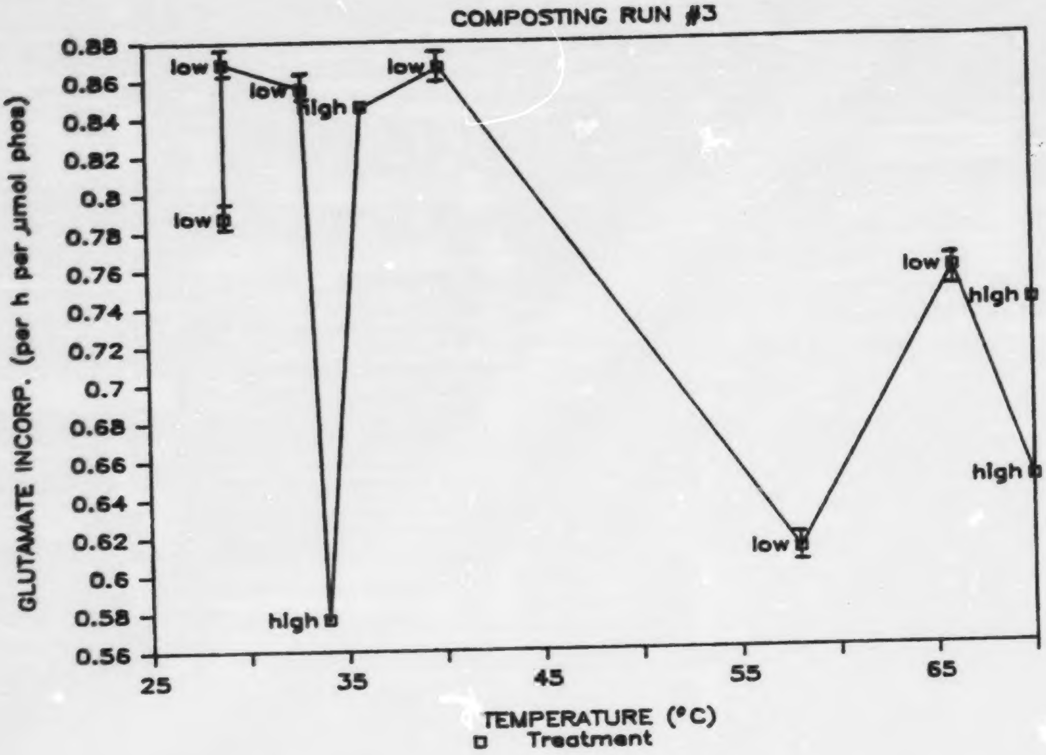

Figure 4. Microbial activity, measured as the rate of [^{14}C]glutamate incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #3. Activity values shown are the mean ± one standard deviation (n=3).

Table 7: Sampling temperature, moisture content, lipid phosphate biomass, and microbial activity (rates of C-14 labled acetate, glucose or glutamate incorporation into lipids per gram dry wt. of compost or per umol lipid phosphate biomass) of cow manure compost samples taken from various depths in either the low-temperature pile or the high-temperature pile during composting run #4. Values shown for dry weight (n=4), lipid phosphate (n=3), and microbial activity (n=3) are means, standard deviations (S.D.), and coefficients of variance (C.V.).

Composting Run #4 Starting Date: 25 August 1981

Day	Treatment (temp.)	Depth (cm)	Temp. (C)	Dry Wt. (%)*	Lipid Ph. (umol/g)	S.D.	C.V.	Acetate (per g)	Glucose (per g)	Glute.+ (per g)	Acetate (per PO4)	Glucose (per PO4)	Glute.+ (per PO4)
1	start	15	50	62.17	2.890	0.046	1.57	0.1701			0.0647		
	start	80	55	58.44	2.630	0.180	6.85	0.1701			0.0647		
6	low	15	43	62.37	2.109	0.266	12.59	0.0955	0.1462	0.1134	0.0307	0.0694	0.0538
	low	80	39	68.23	1.620	0.027	1.65	0.1782	0.1495	0.3920	0.1100	0.0923	0.2420
	high	15	70	56.64	1.498	0.086	5.72	0.0417	0.0961	0.0464	0.0279	0.0642	0.0310
	high	80	67	49.25	1.432	0.058	4.07	0.0624	0.0714	0.1100	0.0436	0.0499	0.0768
10	low	15	34	67.93	1.818	0.069	3.77	0.3395	0.4810	0.6050	0.1868	0.2646	0.3328
	low	80	39	67.59	1.925	0.156	8.11	0.3267	0.6225	0.6258	0.1697	0.3234	0.3251
	high	15	51	64.40	1.682	0.115	6.84	0.4649	0.7419	0.8243	0.2764	0.4411	0.4901
	high	80	44	64.40	1.611	0.164	10.18	0.1219	0.4743	0.6386	0.0757	0.2944	0.3964
15	low	15	38	66.60	1.382	0.018	1.27	0.6986	0.4519	0.4326	0.5055	0.3270	0.3130
	low	80	35	61.27	1.619	0.206	12.71	1.2300	1.1296	0.6740	0.7597	0.6977	0.4163
	high	15	72	54.46	1.279	0.157	12.29	0.4861	0.1916	0.4709	0.3801	0.1498	0.3682
	high	80	65	61.80	1.294	0.141	10.90	0.3084	0.1248	0.3005	0.2383	0.0964	0.2322

* C.V. < 3.60 + Glute. = Glutamate

Table 8: Mean composting pile temperatures (C), taken from the thermocouples, during run #4.

Time (Days)	Low Temp. Pile	High Temp. Pile
1	59	63
6	42	65
10	*	*
15	42	64

* Turning of the piles

Table 9: Matrix of correlation coefficients (r) for parameters (Table 7) measured during composting run #4.

Correlation Matrix: Run #4 ++

	Lipid Phosphate (umol/g)	Glutamate Incorp. (per PO4)	Glucose Incorp. (per PO4)	Acetate Incorp. (per PO4)	Glutamate Incorp. (per g)	Glucose Incorp. (per g)	Acetate Incorp. (per g)	Dry Weight (%)	Depth (cm)	Treatment (pile temp.)	Time (days)
Temperature	-0.2387	-0.3649	-0.5374 *	-0.2785	-0.4666	-0.5839 *	-0.3829	-0.8142 ***	-0.0768	0.7550 ***	-0.0801
Time	-0.7721 ***	0.7836 ***	0.6685 ***	0.7901 ***	0.7052 ***	0.6169 **	0.7106 ***	0.1565	0.0000	0.2446	
Treatment	-0.5668 *	0.1614	-0.0812	-0.1132	0.0769	-0.1174	-0.2435	-0.5377 *	0.0000		
Depth	-0.0782	0.0568	0.0688	0.0222	0.0781	0.0740	-0.0137	-0.0481			
Dry Wt.	0.1357	0.4330	0.3905	0.1593	0.5118	0.4411	0.2319				
Acetate/g	-0.2758	0.6552 **	0.8149 ***	0.9847 ***	0.6609 *	0.7573 **					
Glucose/g	-0.3082	0.8281 ***	0.9908 ***	0.7057 ***	0.8707 ***						
Glutamate/g	-0.4779	0.9838 ***	0.8576 ***	0.5990 *							
Acetate/PO4 +	-0.3866	0.6678 ***	0.7776 ***								
Glucose/PO4 +	-0.3136	0.8368 ***									
Glutamate/PO4 +	-0.5264										

+ arcsine tranformed for ratio correlations with lipid phosphate

++ using a ln transformation for the microbial activity measures

*** $p < 0.01$

** $p < 0.02$

* $p < 0.05$

$n = 14$

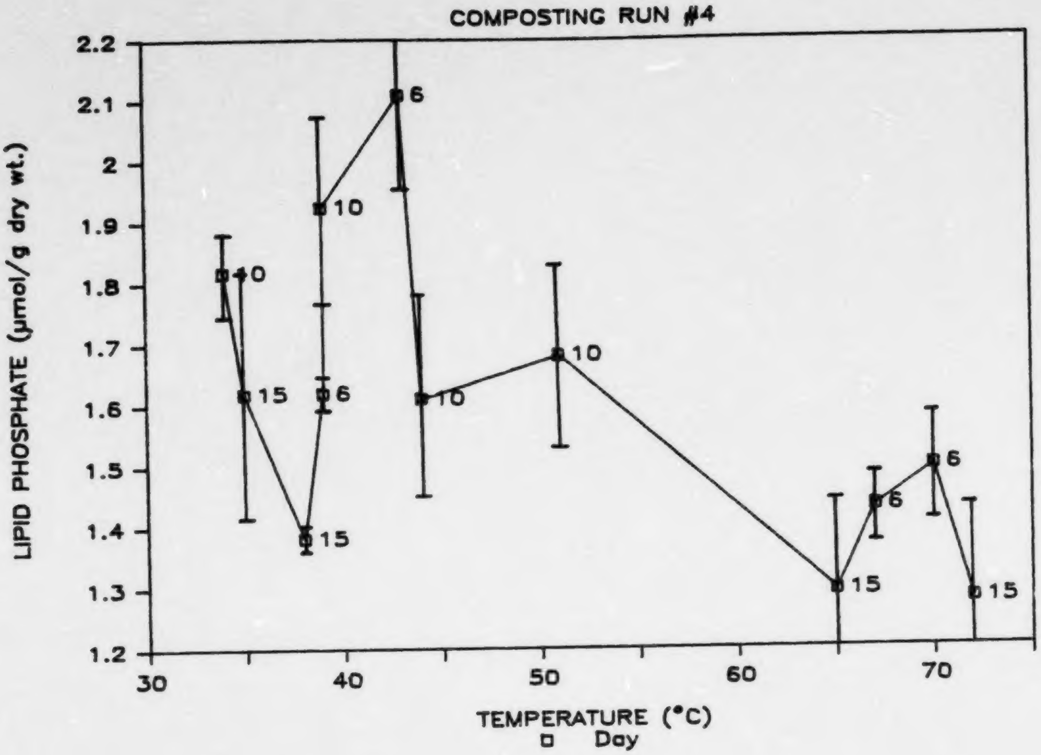

Figure 5. Microbial biomass, measured as the lipid phosphate concentration, in response to temperature in composting cow manure on various days (indicated by the numbers associated with the data points) after the beginning of composting run #4. Biomass values are shown as the mean ± one standard deviation (n=3).

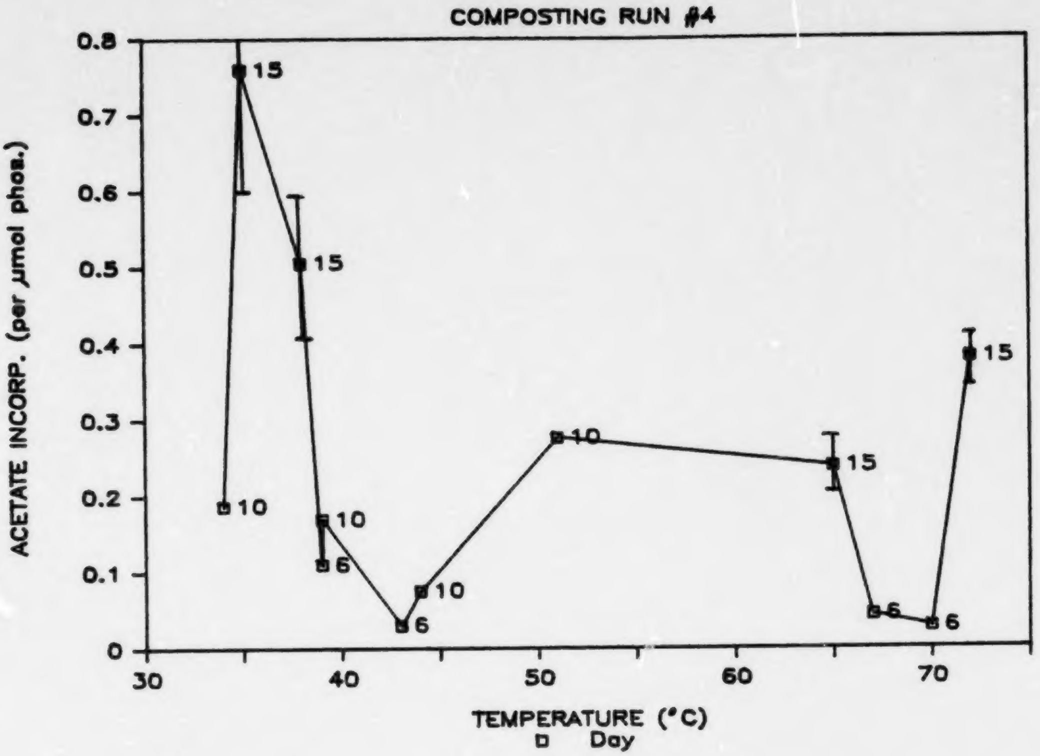

Figure 6. Microbial activity, measured as the rate of [^{14}C]acetate incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #4. Activity values shown are the mean ± one standard deviation (n=3).

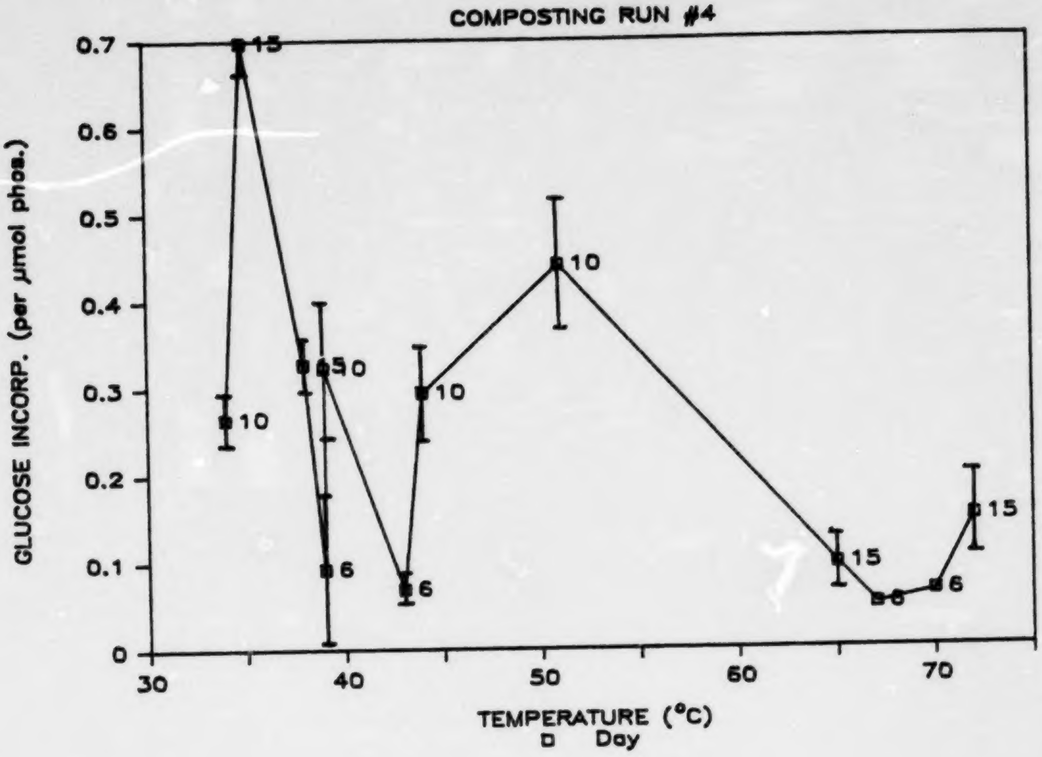

Figure 7. Microbial activity, measured as the rate of [^{14}C]glucose incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #4. Activity values shown are the mean ± one standard deviation (n=3).

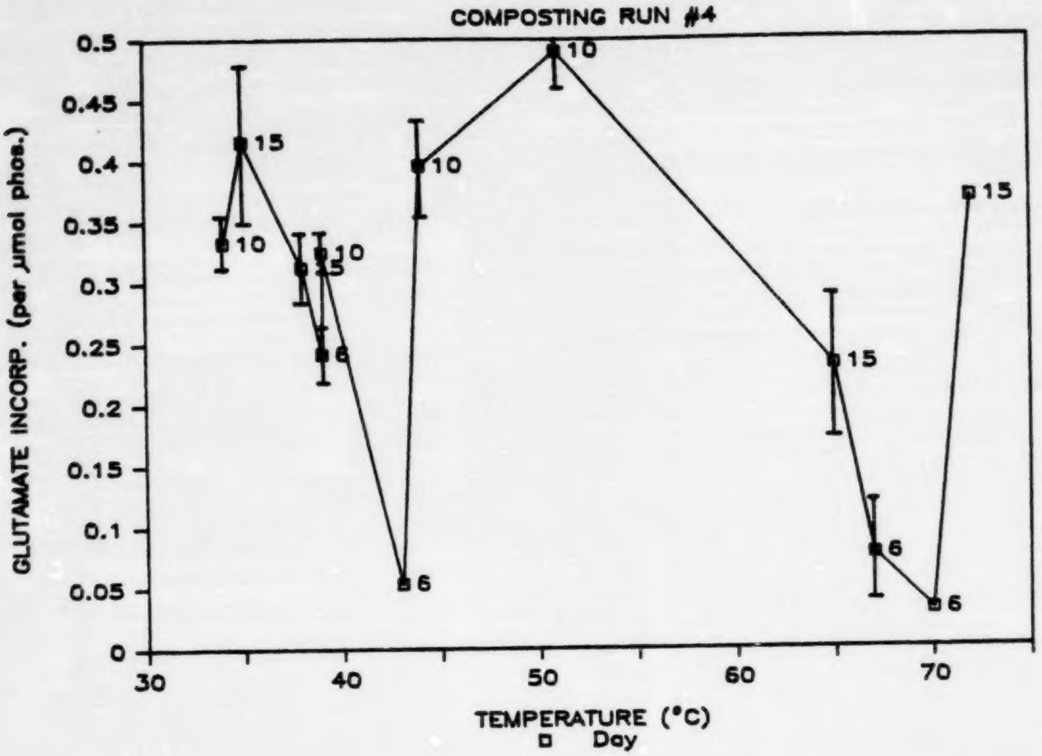

Figure 8. Microbial activity, measured as the rate of [^{14}C]glutamate incorporation into lipid per hour per µmol of lipid phosphate biomass, in response to sampling temperature in composting cow manure taken from either a low-temperature pile (low) or from a high-temperature pile (high) during composting run #4. Activity values shown are the mean ± one standard deviation (n=3).

COMPOSTING RUN #5

Samples were taken on only one day during composting run 5, and were so few that no trends or conclusions could be derived from the data. Therefore, run 5 will not be discussed in the remainder of the report.

COMPOSTING RUN #6

The remainder of the composting runs discussed in this report were performed with sewage sludge obtained from Akron, Ohio.

This experiment was designed to examine the effects of time, temperature, and, to a certain extent, the effects of sampling depth, moisture content and pH, on microbial biomass and activity in composting sewage sludge. The sampling temperature, depth, dry weight and pH are shown in Table 10 for each sample taken during run 6. A correlation matrix of these parameters is given in Table 11. The dry weight and pH of the samples increased over time (Figures 9-10), but the pH also declined with increasing temperature (Figure 11).

The samples taken from each sampling depth on a particular sampling day were pooled for microbiological analysis. Table 12 shows these results, along with the mean sampling temperatures, dry weights and pH values of the samples which were pooled. The incubation temperatures ("Inc. temp.") were set to be near the mean sampling temperature whenever possible. The correlation matrix for these data is given in Table 13. Again, it appears that microbial biomass (Figure 12) and activity (Figures 13-15) significantly declined with increasing sampling temperatures, and that no consistant changes with regard to depth or dry weight could be found. The maximal activity was found in the 35-40°C temperature range (except for on day 0), and activity fell off rapidly at higher temperatures. The mean sample pH and dry weight significantly increased over time, as found previously, but were not related to the mean sampling temperature in this case. However, in a partial correlation of temperature and pH controling for time and dry weight, the correlation coefficient was -0.4105 (n.s.), as compared with the zero-order correlation of -0.0323, indicating that the relationship between pH and temperature may have been masked by the effects of time and dry weight in this case.

COMPOSTING RUN #7

The composting pile was divided into a low- and a high-temperature section, as in runs 3 and 4. This experiment was designed primarily to examine the effects of temperature on the composting microbiota over time, and to compare the rates of microbial substrate incorporation and mineralization under various conditions. The sampling temperatures, dry weights, and pH values

Table 10: Sampling temperature, moisture content, and pH of sewage sludge compost samples taken at various depths during composting run #6. Values shown for dry weight are the means of four replicates. The mean compost pile temperatures (C) are from the thermocouple probes.

Composting Run #6

Day	Pile Temp.	Depth (cm)	Temp. (°C)	Dry Wt. (%)	pH
0	11	15	5	40.36	6.70
			6	30.93	6.40
			8	37.02	6.70
			10	42.94	6.52
		80	4	35.57	6.40
			11	33.71	6.70
			15	39.18	6.85
			17	32.95	6.85
4	39	15	10	29.07	6.89
			38	30.30	7.72
			38	36.58	7.97
			72	31.60	7.15
		80	20	39.20	7.15
			32	36.01	7.41
			42	34.23	6.81
			66	36.84	6.42
8	58	15	50	32.05	8.15
			67	31.85	7.15
			68	33.75	7.95
			80	36.16	7.79
		80	43	39.25	8.00
			62	39.16	6.00
			72	37.47	7.09
			73	37.47	7.09
* 13	55	15	27	44.18	7.78
			41	39.40	7.80
			55	37.28	6.85
			73	36.20	7.90
		80	41	39.64	8.05
			56	39.92	6.70
			65	36.61	6.99
			70	37.99	7.40
** 20	48	15	34	39.52	8.49
			36	41.14	8.38
			37	41.10	8.35
			37	40.89	8.49
		80	26	41.69	8.25
			27	41.46	8.30
			29	40.97	8.60
			31	41.16	8.15
25	57	15	41	37.50	8.21
			44	40.88	8.31
			45	37.95	8.32
			54	38.39	7.06
		80	39	43.50	7.26
			41	39.58	8.16
			41	41.28	8.18
			56	42.20	7.26

* Compost pile was turned on day 11.

** Compost pile was turned on day 18.

Table 11: Matrix of correlation coefficients (r) for physical-chemical parameters (Table 10) from composting run #6.

Correlation Matrix: Run #6 ++

	pH	Dry Wt.	Depth	Time
Temperature	-0.4611 ***	-0.2953	-0.0223	-0.2294
Time	0.5571 ***	0.6800 ***	0.0000	
Depth	-0.2795	0.3402 *		
Dry Wt.	0.3587 **			

++ excluding day 0

*** $p < 0.01$

** $p < 0.02$

* $p < 0.05$

$n = 40$

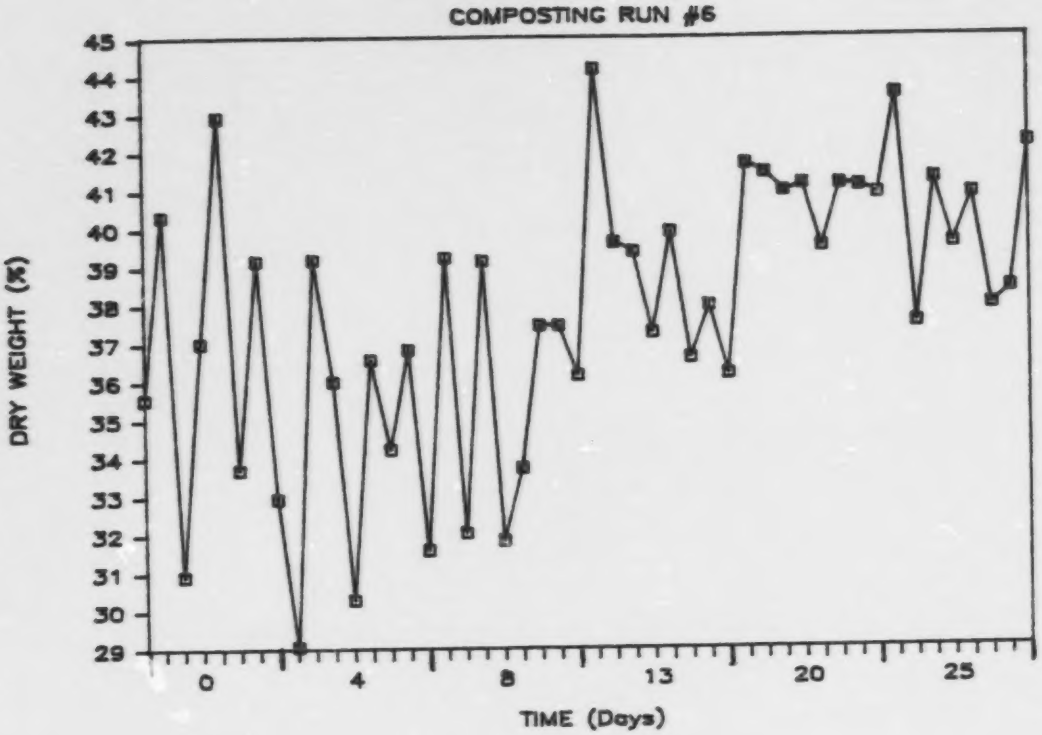

Figure 9. Percent dry weight (Table 10) of composting sewage sludge over time during composting run #6. The samples taken on the same sampling day are seperated along the horizontal axis for clarity. Values are the mean (n=4).

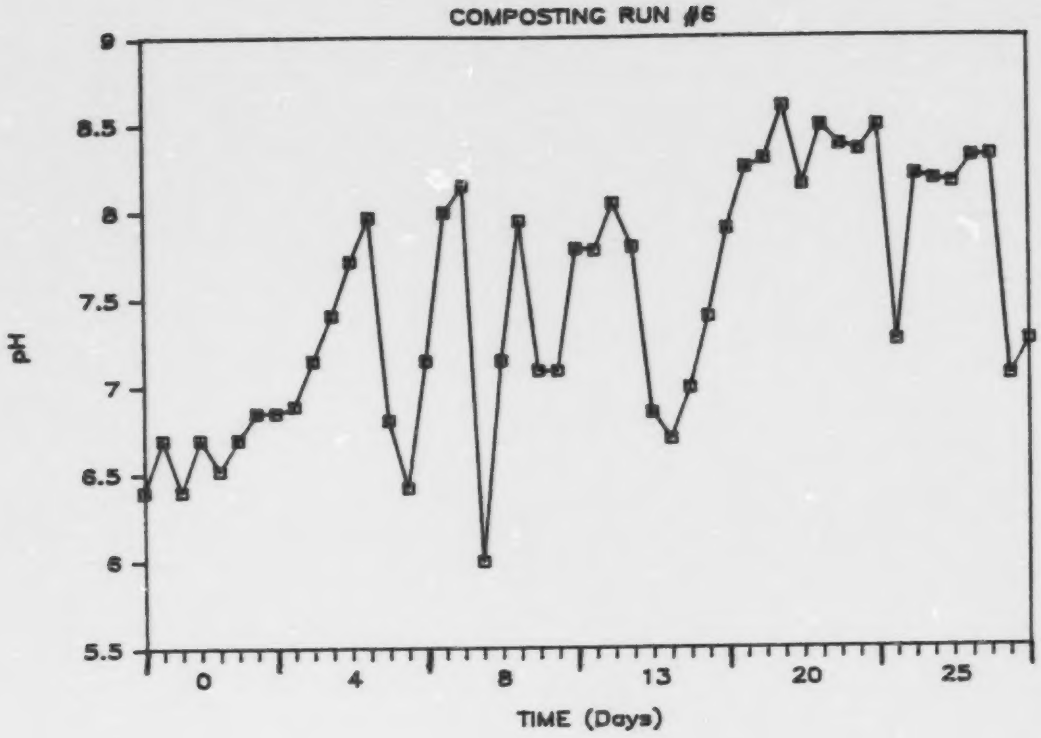

Figure 10. The pH values (Table 10) of composting sewage sludge over time during composting run #6. The samples taken on the same sampling day are seperated along the horizontal axis for clarity (n=1).

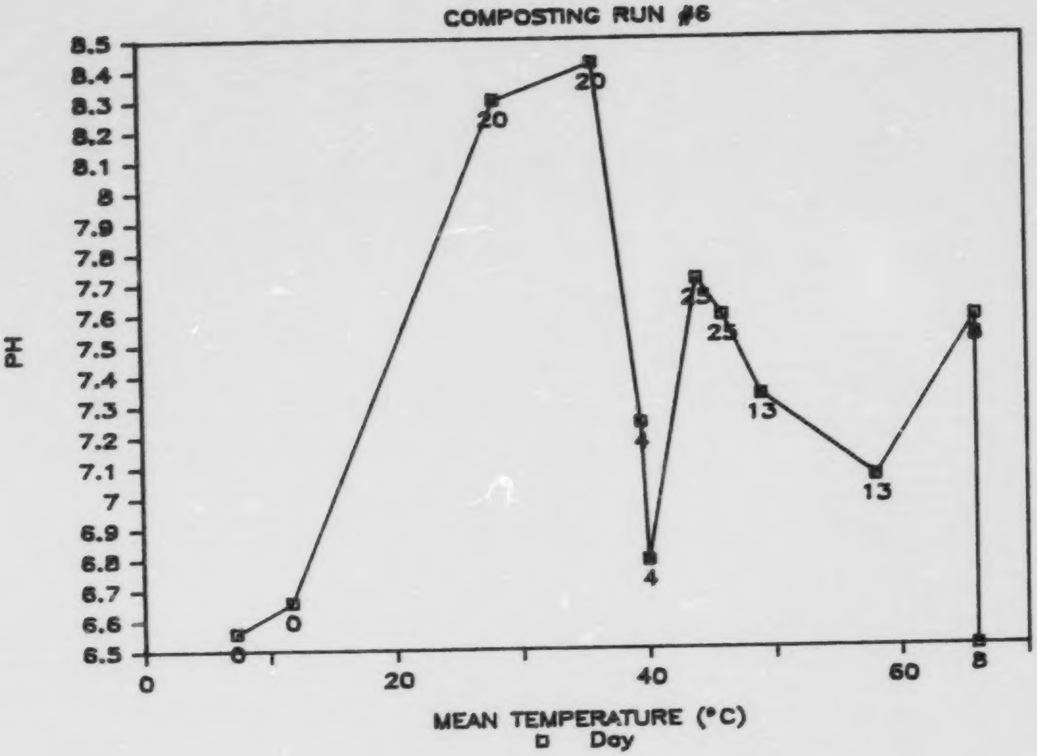

Figure 11. The mean pH values (Table 12) of composting sewage sludge in response to mean sampling temperature during composting run #6.

Table 12: Lipid phosphate biomass and microbial activity (rates of C-14 labeled acetate, glucose, or glutamate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) of sewage sludge compost samples pooled by depth during composting run #6. Mean sampling temperatures, dry weights and pH values are from the individual samples which were pooled (Table 10). The incubation temperatures for the microbial activity assays are also given. Values shown for lipid phosphate and microbial activity are the means (n=3).

Composting Run #6 Starting Date: 22 January 1982

Day	Sample Depth (cm)	Mean Temp. (°C)	Inc. Temp. (°C)	Mean Dry Wt. (%)	Mean pH	Pooled Lipid Ph. (µmol/g)	Acetate Incorp. (per g)	Glucose Incorp. (per g)	Glute.+ Incorp. (per g)	Acetate Incorp. (per PO4)	Glucose Incorp. (per PO4)	Glute.+ Incorp. (per PO4)
0	15	7	20.0	37.81	6.56	4.673	7.7780	5.3020	3.9160	1.6640	1.1350	0.8380
	80	12	20.0	35.35	6.66	5.568	0.4775	6.0530	4.1034	1.4762	1.0870	0.7369
4	15	40	39.5	31.89	7.24	5.300	1.9039	2.6300	2.4351	0.3592	0.4963	0.4595
	80	40	39.5	36.67	6.79	3.400	2.1840	4.6450	3.8070	0.6424	1.3660	1.1198
8	15	66	62.0	33.45	7.58	2.663	0.0876	0.3280	0.4166	0.0329	0.1230	0.1564
	80	66	66.0	37.93	6.50	1.993	0.0659	0.6320	0.1920	0.0331	0.3170	0.0963
* 13	15	49	50.0	39.26	7.33	1.661	0.1927	0.2851	0.3801	0.0920	0.1362	0.1816
	80	58	57.0	38.54	7.06	1.688	0.0740	0.2151	0.2756	0.0345	0.1003	0.1284
** 20	15	36	35.0	40.66	8.42	3.931	4.4350	3.1263	2.2216	1.1284	0.8260	0.5652
	80	28	30.0	41.32	8.30	3.623	0.9569	1.6215	2.3050	0.2641	0.4476	0.6362
25	15	46	46.0	38.68	7.59	2.316	0.1318	0.8789	0.4038	0.0569	0.3796	0.1744
	80	44	44.0	41.64	7.71	2.297	0.1835	0.9811	0.4570	0.0799	0.4272	0.1990

* Compost pile was turned on day 11.

** Compost pile was turned on day 18.

Table 13: Matrix of correlation coefficients (r) for the pooled samples assayed during composting run #6 and the mean values for the physical-chemical parameters (Table 12).

Correlation Matrix: Run #6 ++

	Pooled Lipid Ph. (per g)	Glutamate Incorp. (per PO4)	Glucose Incorp. (per PO4)	Acetate Incorp. (per PO4)	Glutamate Incorp. (per g)	Glucose Incorp. (per g)	Acetate Incorp. (per g)	Mean pH	Mean Dry Wt. (%)	Depth (cm)	Time (days)
Mean Temp.	-0.7937 ***	-0.8000 ***	-0.7671 ***	-0.8460 ***	-0.8671 ***	-0.8514 ***	-0.6648 **	-0.0323	-0.0893	0.0233	0.2237
Time	-0.5380	-0.4566	-0.4210	-0.5032	-0.5202	-0.4964	-0.3271	0.7650 ***	0.7177 ***	0.0000	
Depth	-0.1256	0.1045	0.1191	-0.1031	0.0265	0.0418	-0.3398	-0.2313	0.2802		
Dry Wt.	-0.4436	-0.1014	-0.0607	-0.0903	-0.2153	-0.1848	-0.0148	0.4523			
pH	-0.1126	-0.1253	-0.2585	-0.2046	-0.1313	-0.2294	-0.0063				
Acetate/g	0.6350 **	0.7501 ***	0.7258 ***	0.7903 ***	0.7631 ***	0.7504 ***					
Glucose/g	0.8689 ***	0.9140 ***	0.9631 ***	0.9151 ***	0.9561 ***						
Glutamate/g	0.8840 ***	0.9642 ***	0.8890 ***	0.8735 **							
Acetate/PO4 +	0.7689 ***	0.8073 ***	0.8621 ***								
Glucose/PO4 +	0.6464 *	0.9117 ***									
Glutamate/PO4 +	0.7859 ***										

+ arcsine transformed for ratio correlations with lipid phosphate

++ microbial activity measures were ln tranformed

*** $p < 0.01$

** $p < 0.02$

* $p < 0.05$

$n = 12$

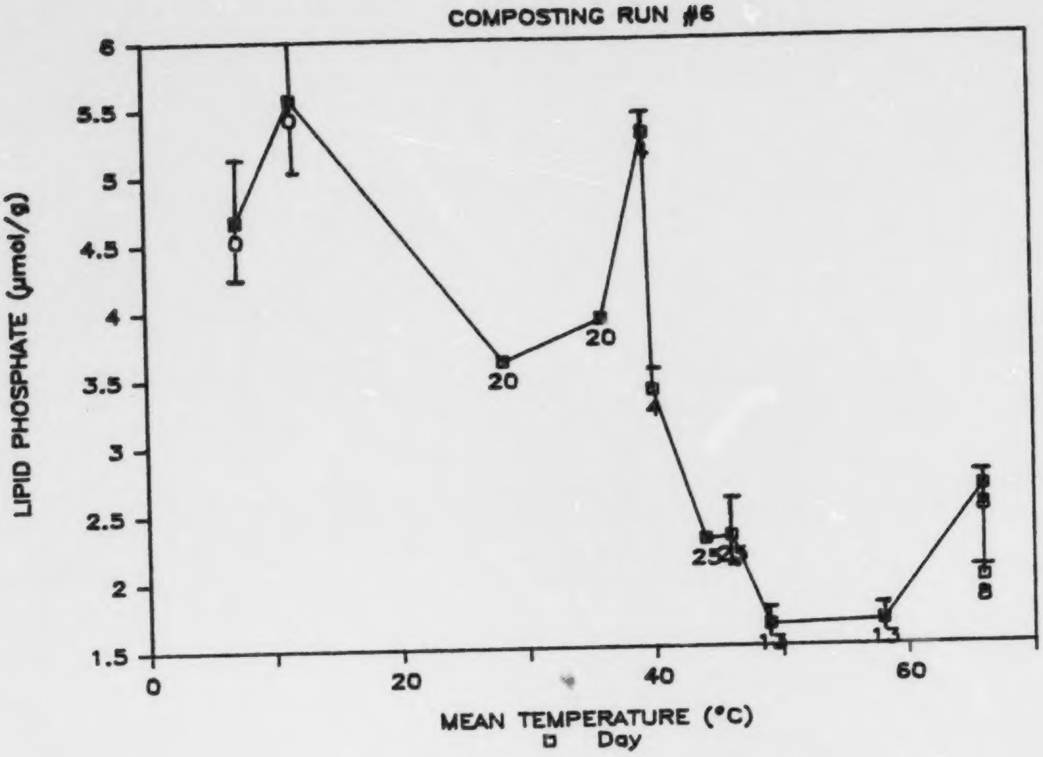

Figure 12. Microbial biomass, measured as the lipid phosphate concentration, of pooled sewage sludge compost samples, in response to the mean sampling temperature of the pooled samples, during composting run #6. Sampling days are indicated by the numbers associated with the data points. Biomass values are the mean ± one standard deviation (n=3).

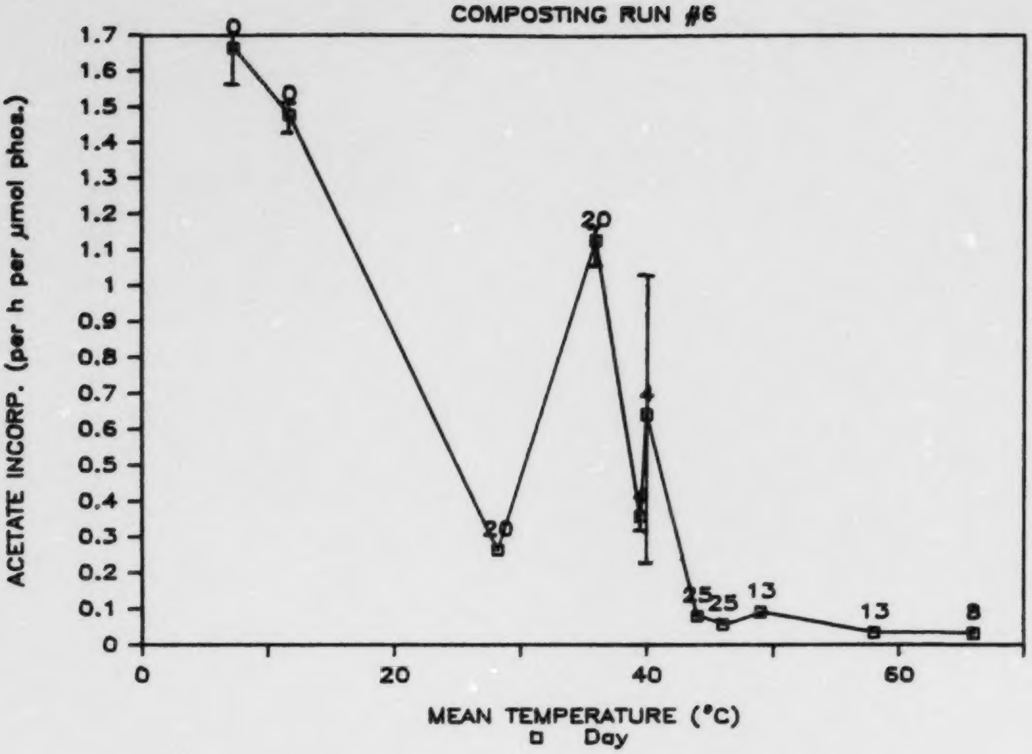

Figure 13. Microbial activity, measured as the rate of [^{14}C]acetate incorporation into lipids per hour per µmol of lipid phosphate biomass x10^{-1}, of pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #6. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean ± one standard deviation of three replicates.

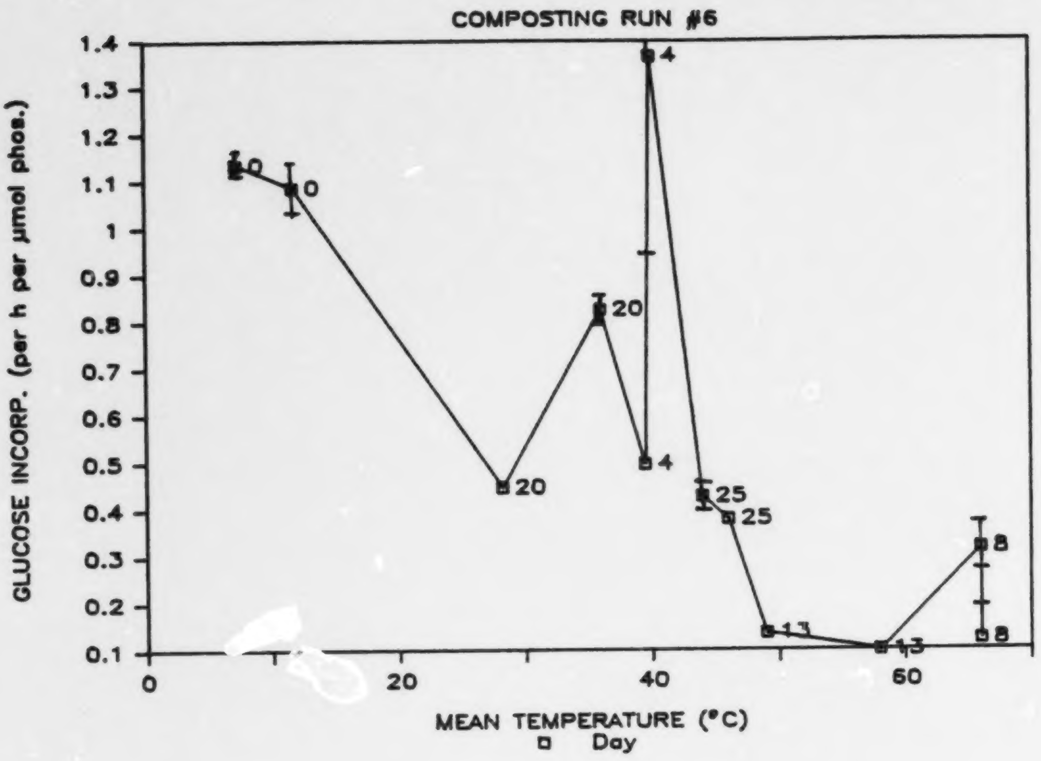

Figure 14. Microbial activity, measured as the rate of [^{14}C]glucose incorporation into lipids per hour per µmol of lipid phosphate biomass x10^{-1}, of pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #6. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean ± one standard deviation of three replicates.

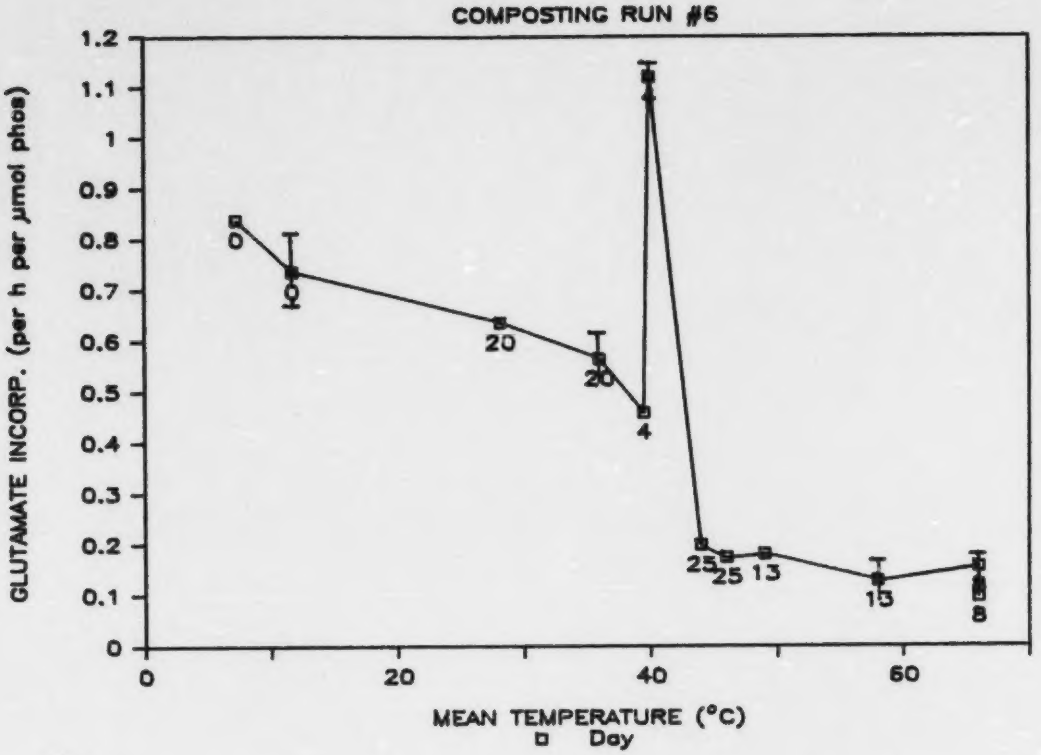

Figure 15. Microbial activity, measured as the rate of [^{14}C]glutamate incorporation into lipids per hour per µmol of lipid phosphate biomass x10^{-1}, of pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #6. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean ± one standard deviation of three replicates.

for each sample are given in Table 14. As was seen previously, the pH and dry weight values of the samples increased over time ($p < 0.01$, Table 15), as did the sampling temperatures. Thus, it is again difficult to strictly seperate the effects of time and temperature in these samples.

The samples from each composting pile (treatment) were pooled on each sampling day for the microbiological assays (Table 16). Lipid phosphate biomass significantly declined with mean sampling temperature ($p < 0.05$, Table 17, Figure 16). Microbial activity, measured as rates of acetate incorporation into microbial lipids and rates of glutamate mineralization to CO_2, also appeared to decrease with increased mean sampling temperature (Figures 17-18), but for this very small sample size the correlations were not significant (Table 17). However, partial correlations between microbial activity per gram of compost and mean sampling temperature controling for lipid phosphate biomass did reveal significant negative relationships ($r = -0.9743$ and $r = -0.9550$, $p < 0.05$ for acetate incorporation and glutamate mineralization rates, respectively). As in previous composting runs, the mean pH of the samples appeared to increase with time and decrease with increasing sampling temperature, but in this case the simple correlations were not statistically significant. First-order partial correlations between time and pH controling for temperature increased the correlation coefficient ($r = 0.7142$, n.s.) considerably. Second order partials formed by also controling for dry weight did not improve upon this correlation very much, indicating that temperature may be the main factor affecting pH aside from time. Partial correlations between mean pH and mean sampling temperature controling for time were also higher ($r = -0.6721$, n.s.) than the corresponding zero-order correlations. Again, adding dry weight as a controled factor did not make much of an improvement, indicating that time and temperature are the best predictors of compost pH. The pH was significantly lower in the high-temperature pile samples than in the low-temperature pile samples (t-test, $p < 0.01$, excluding day 1).

COMPOSTING RUN #8

Samples were taken at several different sampling temperatures on each sampling day (Table 18) in order to obtain a good sample of all of the various microorganisms present at the various temperatures. Samples taken during runs 8, 9 and 10 were all taken near the surface of the pile in an attempt to minimize differences in samples due to variations in moisture content, aeration, etc., so that the effects of temperature on the microbial community might be examined more clearly.

The correlation matrix for the individual samples is shown in Table 19. As found previously, pH increased over time, but this time it also appeared to increase with temperature. This may be

Table 14: Sampling temperature, moisture content, and pH of sewage sludge compost samples taken from either the low-temperature or the high-temperature pile during composting run #7. Values shown for dry weight are the means of four replicates.

Composting Run #7

Day	Treatment (pile)	Temp. (°C)	Dry Wt. (%)	pH
1	start	25	38.20	7.16
		29	36.21	7.08
		29	37.65	7.04
		30	36.18	7.70
7	low	30	35.67	8.99
		32	29.95	8.65
		32	31.22	8.74
		35	36.47	8.68
	high	65	40.54	5.90
		67	61.77	7.50
		67	42.52	7.51
		69	38.74	5.89
* 10	low	46	49.88	8.12
		51	49.24	8.22
		52	44.08	8.15
		54	53.25	8.45
	high	67	50.67	8.45
		68	43.03	8.20
		68	48.85	8.60
		70	42.24	8.22
15	low	42	52.12	7.50
		45	50.28	8.20
		46	50.13	8.11
		48	51.52	7.91
	high	65	49.46	8.70
		66	53.86	8.70
		72	50.44	8.25
		73	49.92	8.20

* Compost pile was turned on day 7.

Table 15: Matrix of correlation cefficients (r) for the physical-chemical parameters (Table 14) from composting run #7.

Correlation Matrix: Run #7

	pH	Dry Wt.	Time
Temperature	0.4391 ##	0.6482 ###	0.6143 ###
Time	0.4924 ###	0.7449 ###	
Dry Wt.	0.7288 ###		

$p < 0.01$
$p < 0.02$
# $p < 0.05$
$n = 29$

Table 16: Lipid phosphate biomass and microbial activity (rates of C-14 labeled acetate incorporation into lipids or glutamate mineralization per gram dry wt. of compost or per µmol lipid phosphate biomass) of sewage sludge compost samples pooled by treatments during composting run #7. Mean sampling temperatures, dry weights, and pH values are from the individual samples which were pooled (Table 14). Values shown for lipid phosphate and microbial activity are the means (n=3).

Composting Run #7 Starting Date: 5 April 1982

Day	Treatment (pile)	Mean Temp. (°C)	Mean Dry Wt. (%)	Mean pH	Pooled Lipid Ph. (µmol/g)	Acetate Incorp. (per g)	Acetate Incorp. (per PO4)	Glutamate Mineral. (per g)	Glutamate Mineral. (per PO4)
1	start	28	37.06	7.18	3.544	0.3409	0.0962		
7	low	32	33.33	8.75	3.140	1.7280	0.5503	11.3594	3.6174
	high	67	38.39	6.19	1.996	0.1102	0.0552	0.7942	0.3982
* 10	low	51	49.11	8.22	1.936				
	high	68	46.20	8.34	1.910				
15	low	45	51.01	7.84	2.459	2.2850	0.9292	11.3174	4.6024
	high	69	50.92	8.40	1.646	0.2497	0.1516	6.3701	3.8690

* Compost pile was turned on day 7.

Table 17: Matix of correlation coefficients (r) for pooled samples assayed during composting run #7 and mean values for the physical-chemical parameters (Table 14).

Correlation Matrix: Run #7 ++

	Lipid Phosphate (umol/g)	Glutamate Mineral. (per PO4)	Glutamate Mineral. (per g)	Acetate Incorp. (per PO4)	Acetate Incorp. (per g)	Mean pH	Mean Dry Weight	Time
Mean Temp.	-0.9726 *	-0.4852	-0.8305	-0.8303	-0.9006	-0.5467	0.4181	0.2425
Time	-0.4610	0.6904	0.3180	0.3148	0.1862	0.3322	0.9731 *	
Dry Wt.	-0.6132	0.5099	0.1132	0.1530	0.0145	0.1100		
pH	0.4103	0.8471	0.8162	0.5947	0.6081			
Acetate/g	0.7810	0.7517	0.9524 *	0.9900 **				
Acetate/PO4 +	0.4733	0.8017	0.9488					
Glutamate/g	0.6812	0.9552 *						
Glutamate/PO4 +	0.0337							

+ arcsine transformed for ratio correlations with lipid phosphate

++ excluding day 1 and using a ln transform of the microbial activity measures

*** $p < 0.01$

** $p < 0.02$

* $p < 0.05$

$n = 4$

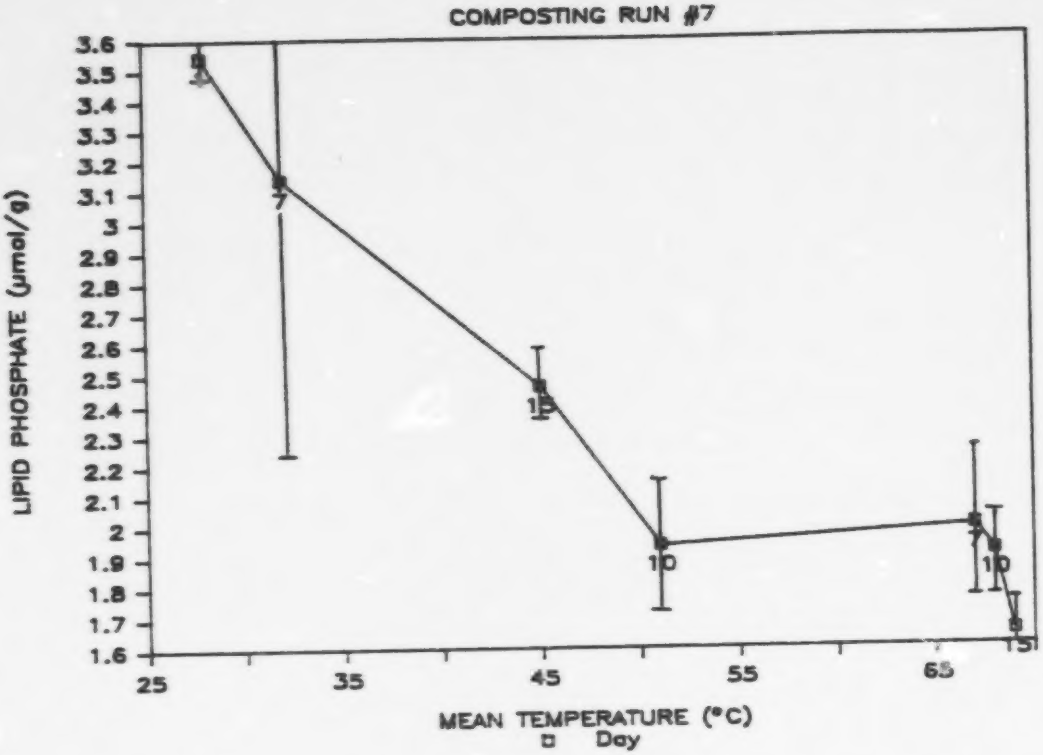

Figure 16. Microbial biomass, measured as the lipid phosphate concentration, of pooled sewage sludge compost samples, in response to the mean sampling temperature of the pooled samples, during composting run #7. Sampling days are indicated by the numbers associated with the data points. Biomass values are the mean ± one standard deviation (n=3).

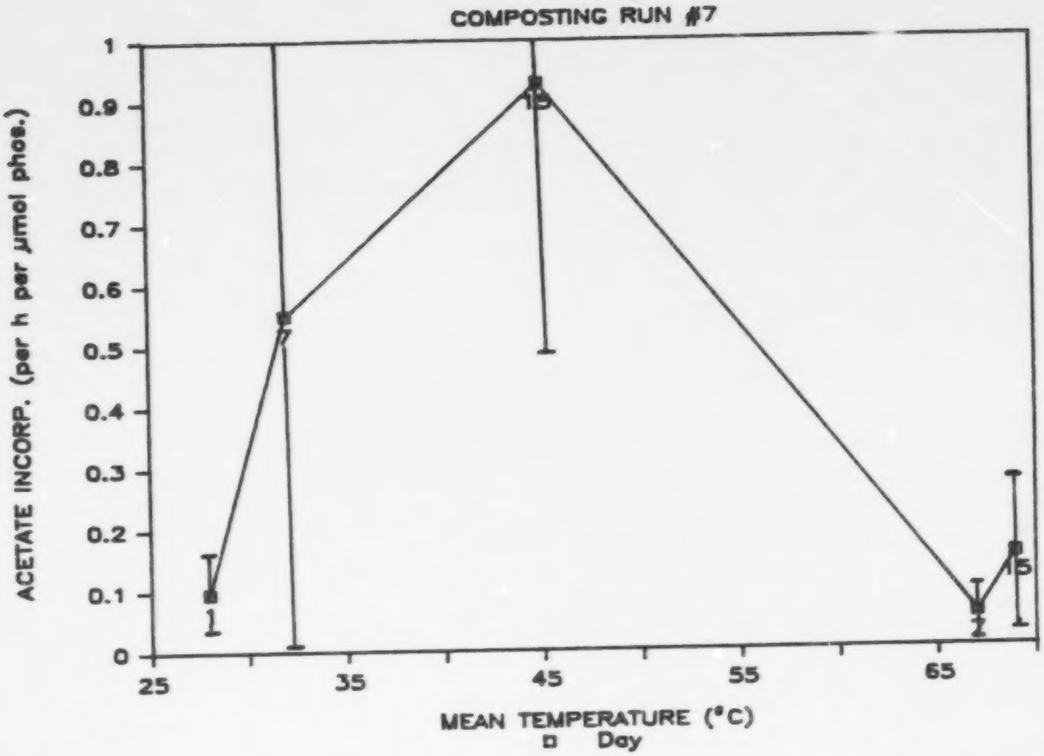

Figure 17. Microbial activity, measured as the rate of [^{14}C]acetate incorporation into lipids per hour per µmol of lipid phosphate biomass, of pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #7. Sampling days are indicated by the numbers associated with the data points. Activity values are the means of three replicates.

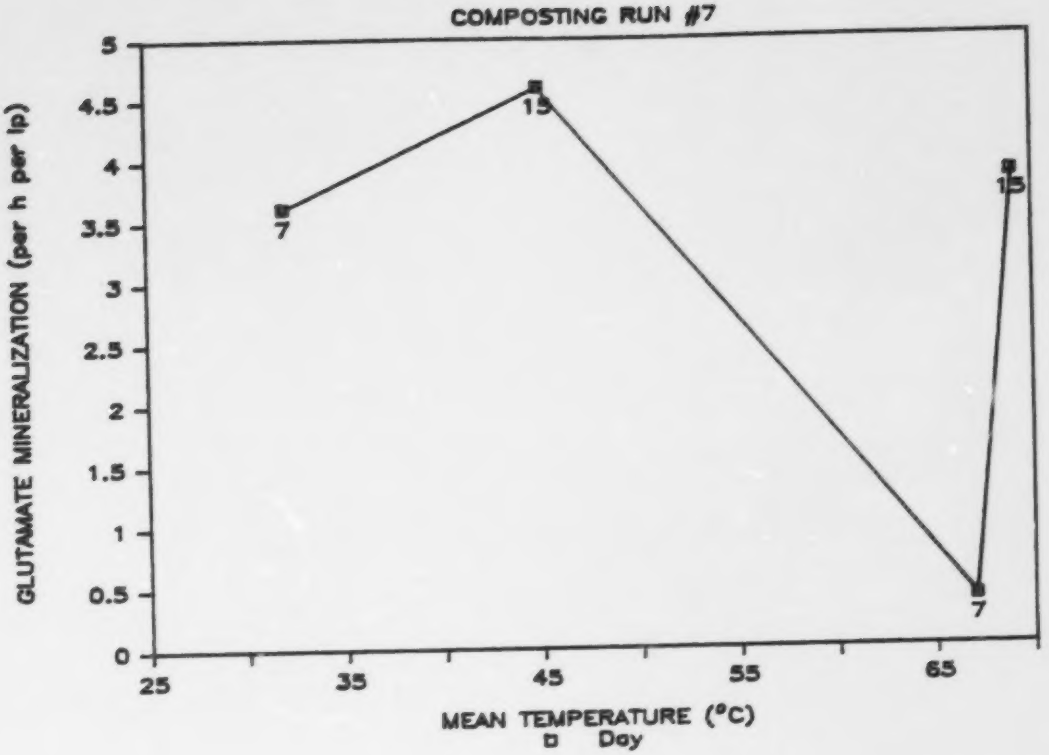

Figure 18. Microbial activity, measured as the rate of [^{14}C]glutamate mineralization to produce $^{14}CO_2$ per hour per µmol of lipid phosphate biomass, of pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #6. Sampling days pooled sewage sludge compost samples in response to the mean sampling temperature of the pooled samples during composting run #7. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean of three replicates.

Table 18: Sampling temperature, moisture content, and pH of sewage sludge compost samples taken during composting run #8. Values shown for dry wt. are the mean of four replicates.

Composting Run #8

Day	Temp. (°C)	Dry Wt. (%)	pH
1	22	45.79	6.85
	22	47.05	6.50
	23	50.93	6.96
	24	52.84	6.70
5	22	59.26	7.90
	35	38.49	8.50
	43	42.24	8.05
	52	45.58	8.60
	64	34.54	8.65
	74	39.69	8.10
* 12	40	44.94	8.50
	47	46.69	8.79
	48	48.91	8.30
	53	42.59	9.00
	61	43.00	9.00
	68	43.43	7.65

* Compost pile was turned on days 7 and 11.

Table 19: Matrix of correlation coefficients (r) for the physical-chemical parameters (Table 18) measured during composting run #8.

Correlation Matrix: Run #8

	pH	Dry Wt.	Time
Temperature	0.6460 ***	-0.6567 ***	0.5902 **
Time	0.7396 ***	-0.1953	
Dry Wt.	-0.4539		

*** p < 0.01
** p < 0.02
* p < 0.05
n = 16

due to the fact that the samples from late in the run (day 12) were taken immediately after the pile had been turned for the second time. Turning tends to homogenize the compost with regard to the temperature gradients in the piles, and thus the values of the slowly-changing parameters, such as pH, may not be truely representative of that sampling temperature until the temperature gradients are reestablished and the parameters have had time to adjust to the new temperatures. In addition, a partial correlation between pH and temperature controling for time and dry weight has a lower correlation coefficient ($r = 0.1010$, n.s.), which is no longer significant. Partial correlations between time and pH controling for temperature and/or dry weight maintained their high level of significance, however, indicating that the correlation between time and pH is very strong. Figure 19 shows that the maximum pH values were found on days 5 and 12 in samples taken from the 50 to 60°C range.

All of the samples taken on a particular sampling day during run 8 were pooled for microbiological analysis. Microbial activities were then determined at several different incubation temperatures (Table 20) in order to determine a temperature optimum for the overall heterotrophic microbial community. Figure 20 shows some typical mineralization (CO_2 production) curves obtained for glutamate during run 8. The slopes of these types of curves were used for the mineralizations rates. Microbial activity declined with increasing incubation temperature on each particular sampling day (Figures 21-22), but the overall correlations were not statistically significant (Table 21). Once again, maximum actvity was found in the 35 to 45°C temperature range. As was found in composting run 7, the first-order partial correlations between temperature and activity per gram of compost, controling for lipid phosphate biomass, revealed significance in all cases ($p < 0.05$).

COMPOSTING RUN #9

This experiment was designed to look specifically at the effects of sampling temperatures and incubation temperatures on the microbial activity of individual samples, as well as pooled samples. As in composting run 8, samples were taken from a variety of different sampling temperatures on each sampling day. However, during run 9, the microbial biomass and activity of each sample was determined (Table 22). In addition, each sample was analyzed for total organic matter (volatile solids), and total protein content, as well as for pH and dry weight. The correlation matrix for these individual samples is presented in Table 23. Unlike some of the previous composting runs, lipid phosphate biomass did not negatively correlate with time or temperature significatly. The fact that pH (Figure 23), dry weight (Figure 24), organic matter (Figure 25), protein (Figure 26), and lipid phosphate (Figure 27) do not correlate well with sampling temperature in these samples allows one to make more

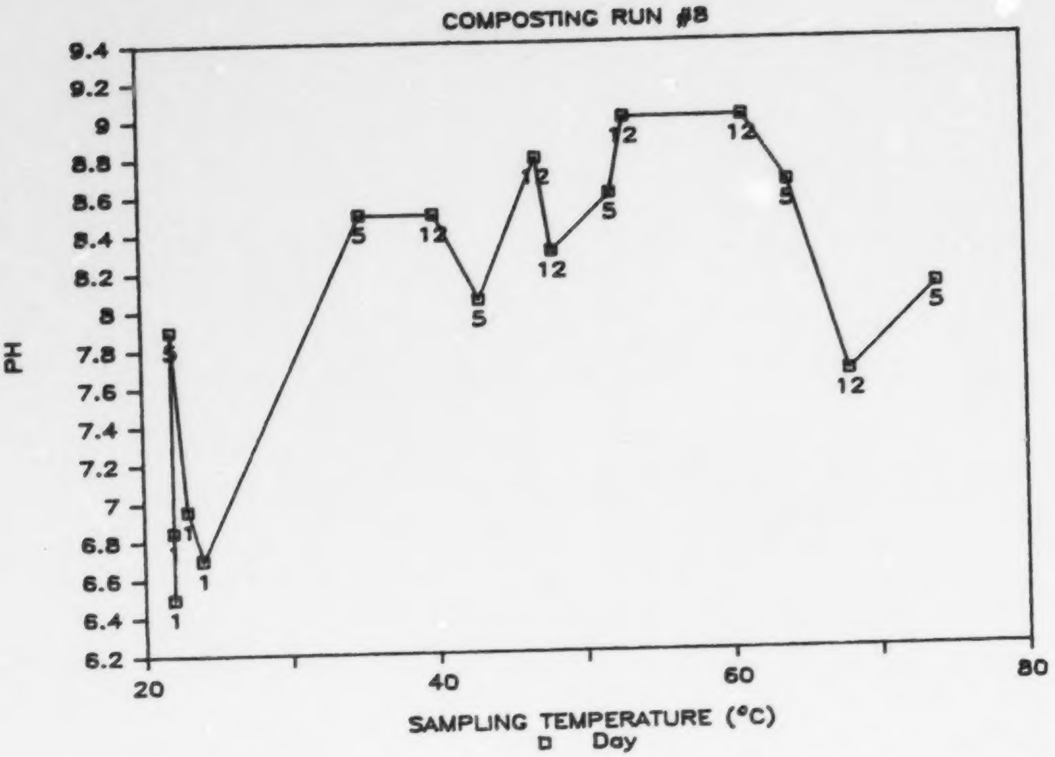

Figure 19. The pH values of sewage sludge compost in response to sampling temperature during composting run #8. The sampling days are indicated by the numbers associated with the data points.

Table 20: Lipid phosphate biomass and microbial activity (rates of C-14 labeled acetate incorporation into lipids or glutamate mineralization to carbon dioxide per gram dry wt. of compost or per µmol lipid phosphate biomass) of sewage sludge compost samples pooled on each sampling day during composting run #8. Mean sampling temperatures, dry weights, and pH values of the individual samples (Table 18) which were pooled are also given. Values shown for lipid phosphate and microbial activity.are the means (n=3).

Composting Run #8 Starting Date: 23 April 1982

Day	Mean Dry Wt. (%)	Mean pH	Pooled Lipid Ph. (µmol/g)	Mean Temp. (°C)	Assay Incubat. Temp.	Acetate Incorp. (per g)	Acetate Incorp. (per PO4)	Glutamate Mineral. (per g)	Glutamate Mineral. (per PO4)
1	49.15	6.72	2.268	23	23	0.1576	0.0695	2.6200	1.2370
5	43.30	8.20	2.169	48	20	0.1474	0.0680	0.3941	0.3418
					35	0.1831	0.0844	0.9558	0.9075
					45	0.1391	0.0641	0.9291	1.0016
					55	0.0429	0.0198	0.1442	0.0917
					65	0.0390	0.0180	0.0186	0.0110
* 12	44.93	8.24	1.434	53	35	3.0820	3.6620	6.5560	7.7950
					40	3.2260	3.8330	6.2650	7.4480
					45	1.0770	1.2800	6.2090	7.3820
					50	0.2670	0.3170	2.3350	2.7760
					55	0.2780	0.3300	3.7000	4.3990

* compost pile was turned on days 7 and 11.

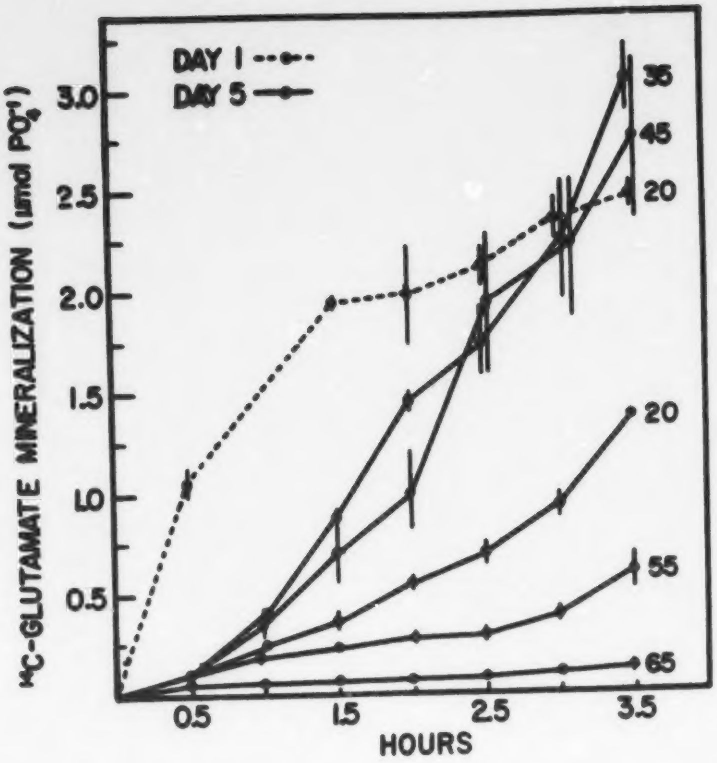

Figure 20. Microbial activity, determined as the amount of $^{14}CO_2$ produced from [^{14}C]glutamate, in composting sewage sludge 1 and 5 days after the beginning of composting run #8. The sampling temperature (and the incubation temperature of the assay) is indicated to the right of each line. Values shown are means ± one standard deviation (n=3).

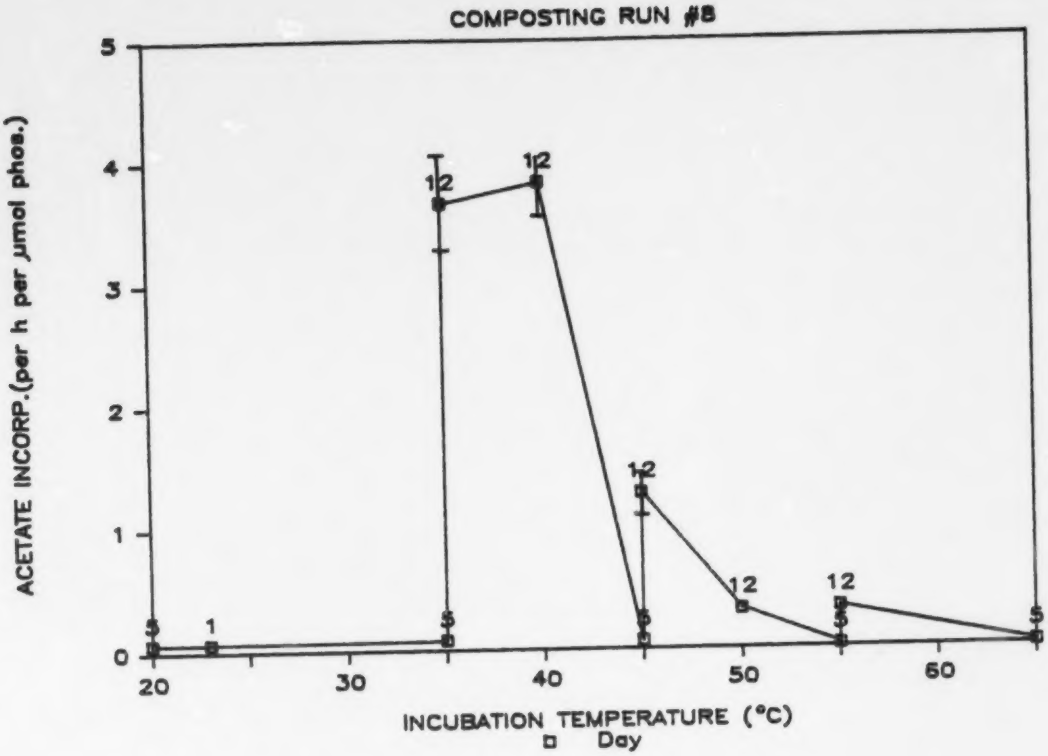

Figure 21. Microbial activity, measured as the rate of [^{14}C]acetate incorporation into lipids per hour per µmol of lipid phosphate biomass, of pooled sewage sludge compost samples in response to the incubation temperature of the assay during composting run #8. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean ± one standard deviation of three replicates.

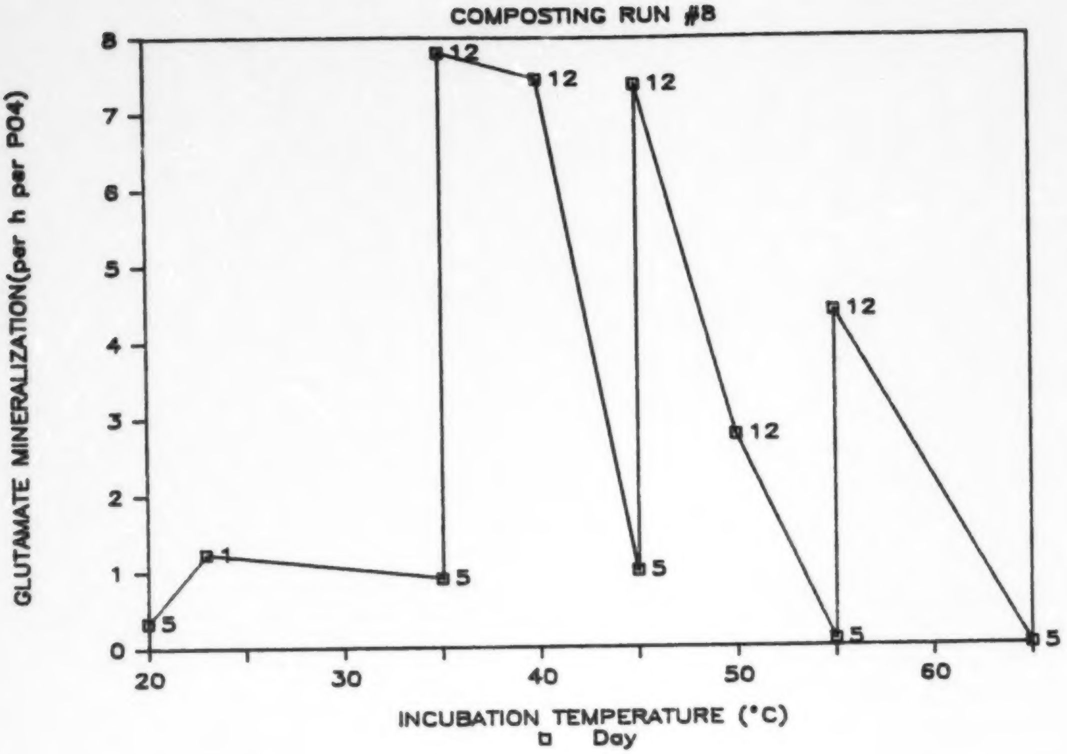

Figure 22. Microbial activity, measured as the rate of [^{14}C]glutamate mineralization to produce $^{14}CO_2$ per hour per µmol of lipid phosphate biomass, of pooled sewage sludge compost samples in response to the incubation temperature of assay during composting run #8. Sampling days are indicated by the numbers associated with the data points. Activity values are the mean of three replicates.

Table 21: Matrix of correlation coefficients (r) for the pooled activity and biomass determinations made during composting run #8, and the incubation temperatures of the assays.

Correlation Matrix: Run #8 ++

	Lipid Phosphate (µmol/g)	Glutamate Mineral. (per PO4)	Glutamate Mineral. (per g)	Acetate Incorp. (per PO4)	Acetate Incorp. (per g)
Incub. Temperature	-0.0412	-0.1590	-0.1826	-0.2605	-0.3070
Acetate/g	-0.6849 #	0.8238 ###	0.8309 ###	0.9977 ###	
Acetate/PO4 +	-0.5952	0.8438 ###	0.8493 ###		
Glutamate/g	-0.9198 ###	0.9994 ###			
Glutamate/PO4 +	-0.8276 ###				

+ arcsine tranformed for ratio correlation with lipid phosphate

++ excluding day 1 and using a ln transform of the microbial activity measures.

$p < 0.01$

$p < 0.02$

# $p < 0.05$

$n = 10$

Table 22: Sampling temperature, moisture content, pH, organic content, total protein, lipid phosphate biomass, and microbial activity (rate of C-14 acetate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) for sewage sludge compost samples taken during composting run #9. Values shown for dry wt. (n=4), pH (n=3), organics (n=4), protein (n=3), lipid phosphate (n=3), and microbial activity (n=3) are the means of the replicates.

Composting Run #9

Day	Sampling Temp. (°C)	Dry Wt. (%)	pH	Total Organics (% of dry)	Total Protein (% of dry)	Lipid Phosphate (µmol/gdw)	Acetate Incorp. (per gdw)	Acetate Incorp. (per PO4)
0	29	41.47	7.02	62.90	2.512	1.759	0.1711	0.0973
	33	41.55	6.95	63.38	2.937	1.759	0.1711	0.0973
	34	40.18	6.95	62.77	2.634	1.759	0.1711	0.0973
	35	40.82	7.00	62.49	2.851	1.759	0.1711	0.0973
3	34	61.85	7.95	64.43	3.801	3.158	11.6256	3.6818
	38	43.06	8.74	62.71	4.169	2.129	0.4653	0.2186
	45	47.30	8.06	62.58	2.947	0.975	0.2503	0.2567
	49	40.24	8.45	61.51	1.906	2.045	0.0456	0.0223
	55	48.98	8.56	62.49	3.411	5.562	0.0620	0.0111
	68	40.94	8.19	64.97	2.992	2.697	0.0508	0.0188
5	44	42.59	7.74	62.85	2.363	3.215	0.0313	0.0097
	50	46.12	8.65	59.58	2.327	2.375	0.0522	0.0220
	60	43.44	7.92	61.67	2.865	2.745	0.0319	0.0116
	65	46.27	6.54	63.93	2.460	1.890	0.0368	0.0195
* 7	40	47.29	7.33	63.08	2.562	0.846	0.0344	0.0407
	45	50.34	8.23	63.02	3.042	0.542	0.1431	0.2641
	50	52.40	8.94	63.22	3.268	0.706	0.1142	0.1618
	55	52.16	8.58	62.46	3.250	0.523	0.0951	0.1819
	60	46.24	7.22	62.66	2.947	1.421	0.0387	0.0272
	68	48.62	7.89	63.02	2.831	0.560	0.0378	0.0676
10	35	49.25	8.56	60.74	3.510	2.217	5.4863	2.4749
	46	50.49	8.83	61.77	3.233	1.594	0.0647	0.0406
	55	40.72	9.07	62.67	3.208	1.017	0.0760	0.0747
	61	46.84	7.55	57.68	2.791	1.073	0.0371	0.0345
	67	47.35	7.35	61.25	2.689	1.717	0.0312	0.0182
** 12	48	48.34	8.73	63.22	2.877	1.569	0.1724	0.1099
	55	44.08	8.80	63.32	2.742	1.028	0.1759	0.1712
	66	48.88	8.13	63.65	2.590	1.331	0.0120	0.0090
	72	50.84	7.91	61.64	2.887	1.155	0.0107	0.0092
14	40	43.59	8.81	62.05	3.367	1.656	2.4942	1.5059
	50	43.33	8.83	62.60	3.515	2.109	0.8713	0.4131
	60	42.79	8.97	61.40	2.900	1.708	0.3912	0.2290
	65	45.71	8.25	59.78	2.864	2.825	0.1097	0.0388
	69	43.47	8.53	61.29	3.015	1.965	0.0311	0.0158
17	44	44.71	8.74	60.70	2.641	2.097	0.9902	0.4722
	51	54.71	8.72	62.42	3.209	1.939	0.2714	0.1399
	55	42.12	8.94	62.39	3.143	2.264	0.1321	0.0583
	60	45.62	8.56	61.15	3.568	2.548	0.1695	0.0665
	66	48.32	8.15	63.14	3.626	2.019	0.0167	0.0083
	75	50.67	7.07	63.12	3.667	1.876	0.0126	0.0067
19	40	42.97	8.65	58.48	3.220	1.130	1.8708	1.6559
	45	43.86	8.57	61.11	3.862	2.764	1.5682	0.5673
	50	41.46	8.82	60.12	4.261	1.204	0.5661	0.4700
	55	46.39	8.76	61.51	3.114	2.572	0.2451	0.0953

* Compost pile was turned on day 5.
** Compost pile was turned on day 11.

Table 23: Matrix of correaltion coefficients (r) for parameters (Table 22) measured on individual compost samples taken from various temperature zones during composting run #9.

Correlation Matrix: Run #9 ++

	Lipid Phosphate	Acetate Incorp./PO4	Acetate Incorp./g	Total Protein	Organic Content	pH	Dry Weight	Time (days)
Temperature	-0.0195	-0.4953 ***	-0.4886 ***	-0.0608	-0.0219	0.0135	0.1337	0.3646 **
Time	-0.0677	0.1325	0.1254	0.3913 ***	-0.4142 ***	0.5160 ***	0.0654	
Dry Wt.	-0.0328	0.2913	0.3021 *	0.2363	0.1911	0.0289		
pH	0.0587	0.2323	0.2147	0.3668	-0.2475			
Organics	0.0164	-0.1095	-0.0697	-0.0021				
Protein	0.1316	0.4198 ***	0.4360 ***					
Acetate/g	0.1826	0.9778 ***						
Acetate/PO4 +	-0.3858 **							

+ arsine tranformed for ratio correlations with lipid phosphate
++ excluding day 19 and using a ln transform for the microbial activity measures
*** $p < 0.01$
** $p < 0.02$
* $p < 0.05$
$n = 44$

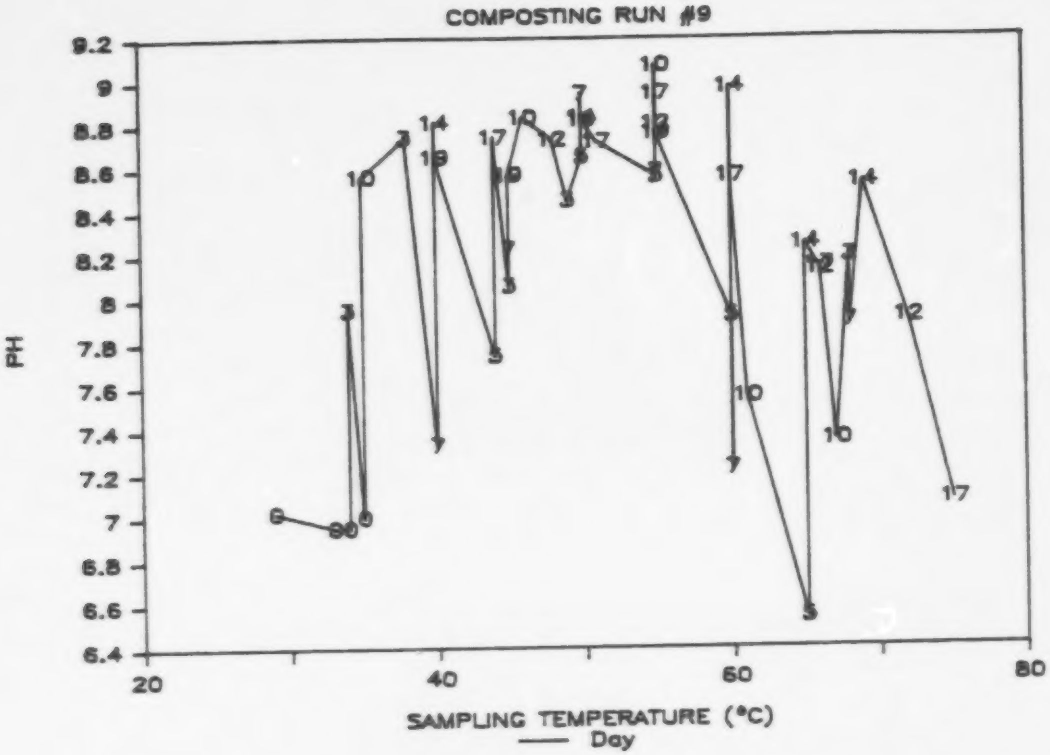

Figure 23. The pH values of sewage sludge compost in response to sampling temperature during composting run #9. The sampling days are indicated by the numbers associated with each data point. The pH values shown are the mean of three replicate determinations.

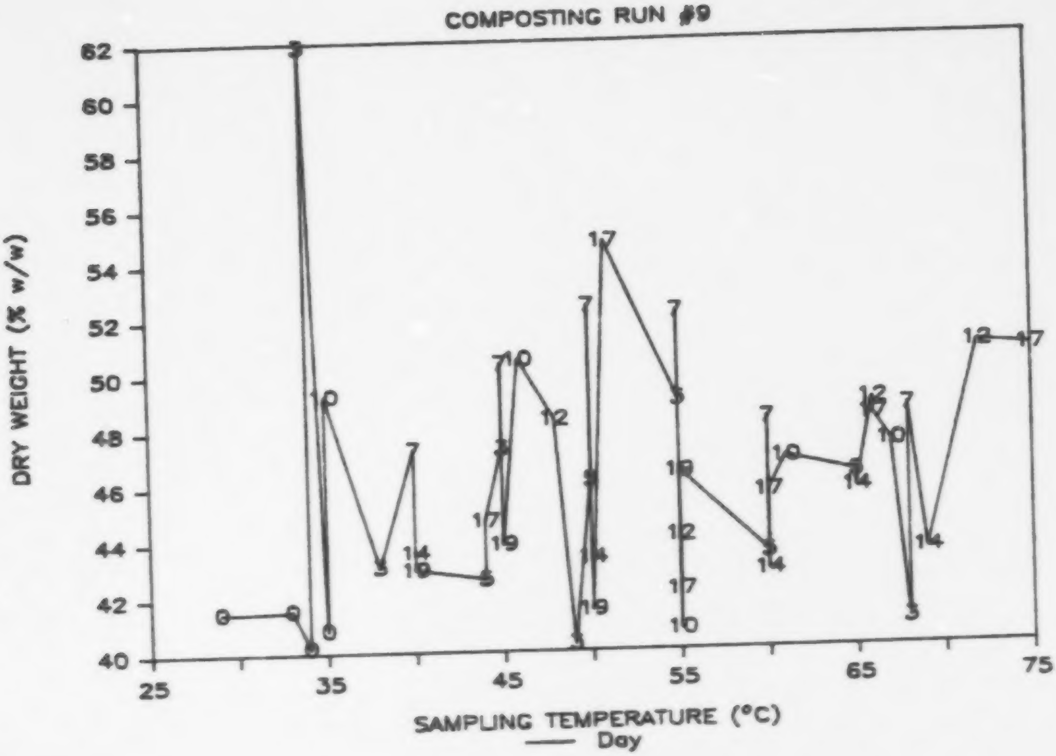

Figure 24. Percent dry weight of sewage sludge compost in response to sampling temperature during composting run #9. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of four replicate determinations.

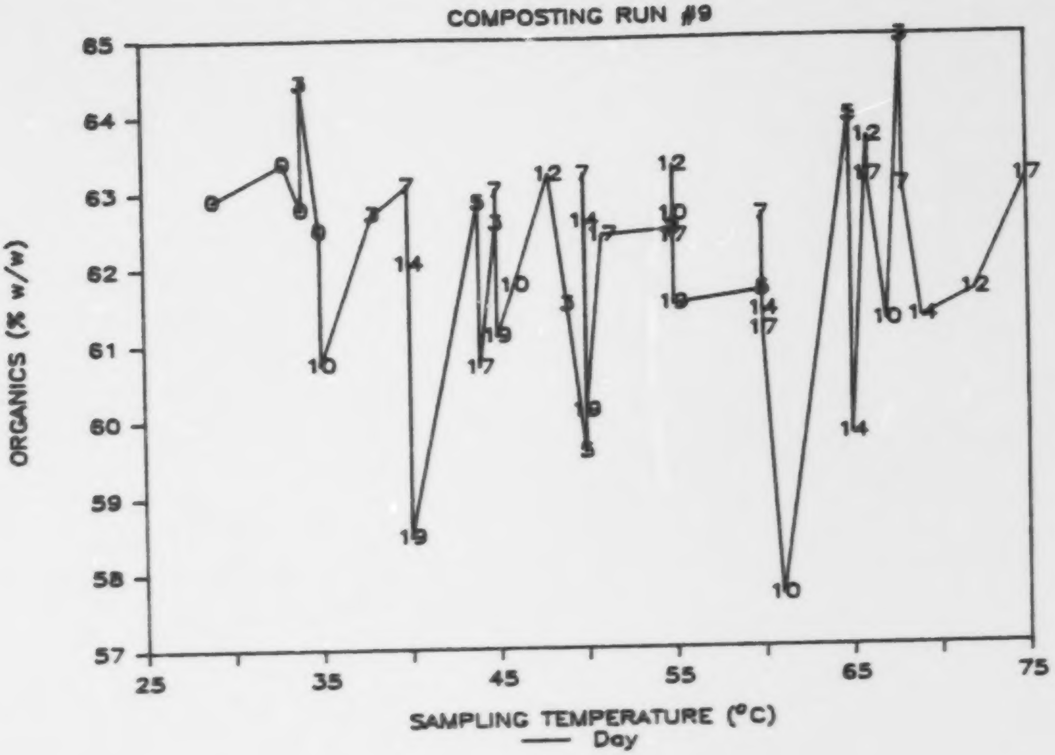

Figure 25. Total organic content of sewage sludge compost in response to sampling temperature during composting run #9. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of four replicate determinations.

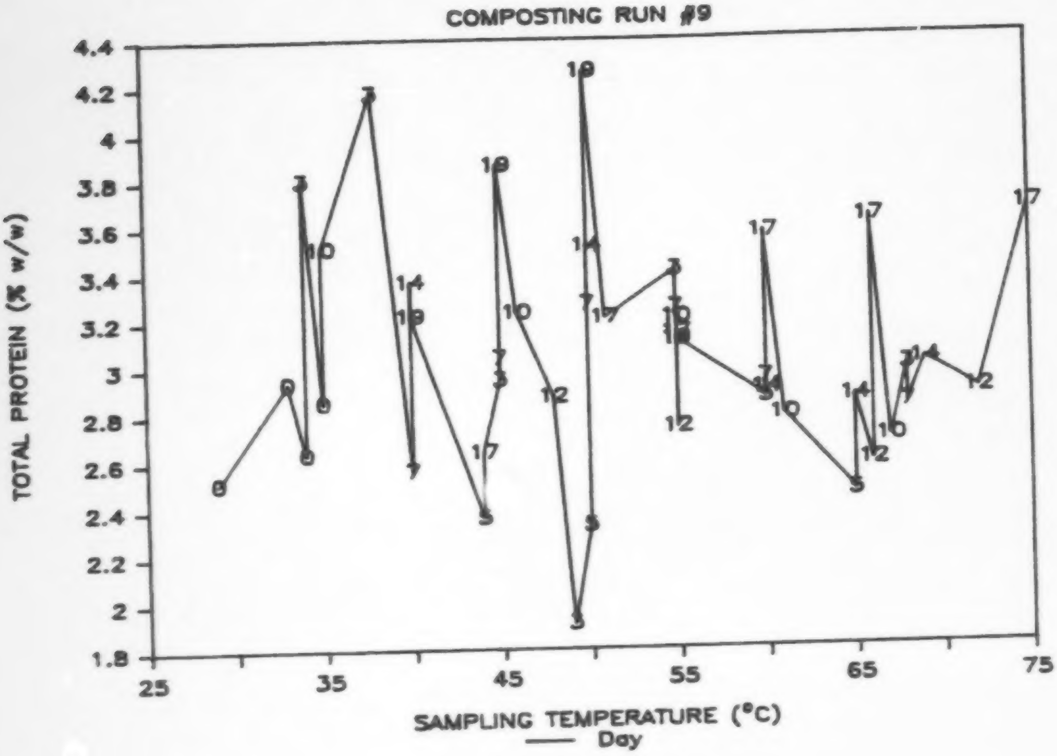

Figure 26. Total protein content of sewage sludge compost in response to sampling temperature during composting run #9. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

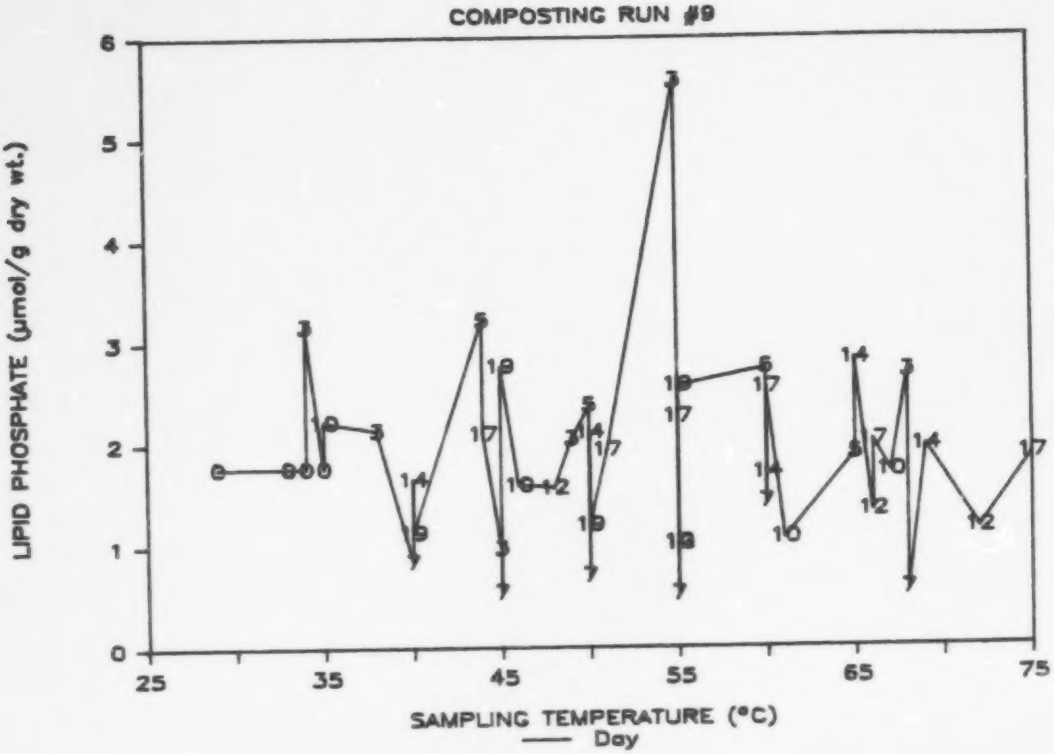

Figure 27. Microbial biomass, determined as the lipid phosphate concentration, of sewage sludge compost in response to sampling temperature during composting run #9. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

clear-cut statements about the effects of temperature on microbial activity, independent of these other factors. However, it should be noted that the pH was generally higher and less variable in samples taken from the 45 to 55°C range. In addition, a partial correlation of pH and temperature controling for time made the correlation coefficient negative (r = - 0.2190, p = 0.158), as had been found in previous runs. Microbial activity (acetate incorporation into lipids) at the sampling temperature per gram of compost (Figure 28) and per unit of lipid phosphate biomass (Figure 29) was highest in the 35 to 45°C range, and was very low at sampling temperatures above 55°C.

During run 9, subsamples of each of these individual samples were also incubated at several different temperatures during the activity assays, in order to determine an optimum temperature for microbial activity in each sample. These types of results are shown in Figure 30 for four of the sampling days during run 9. It can be seen that the microbial communities from the lower sampling temperatures (35 to 45°C) generally had much greater activity than those from the higher-temperature samples (65 to 75°C), usually by as much as an order of magnitude, regardless of the incubation temperature. In addition, the samples from high-temperature regions had flatter temperature-response curves, showing little or no significant response to varied incubation temperature. In contrast, the samples from low-temperature regions generally showed a distinct curvilinear response to varied incubation temperature, with an optimum for activity within the 35 to 50°C range.

The biomass and activity (determined at the mean sampling temperature) of the pooled samples from each sampling day, along with the mean values for sampling temperature, dry weight, pH, organics, protein and lipid phosphate determined from the individual samples prior to pooling are shown in Table 24. The mean compost pile temperature, taken from the 80 temperature probes, is also shown for each sampling day that the probes were in place. Although a negative relationship can again be seen between actvity and temperature (Table 25), these correlations were not statistically significant for this small sample size. The mean compost pile temperatures, dry weights, organic contents, pH values, protein contents and biomasses for each sampling day are shown in Figures 31-36. Note that the mean compost pile temperature remained fairly low until after day 12 of composting. This may be due to a condition of poor aeration which existed until the bottom of the composting bin was cleaned and broken up during the second turn (days 11-12). Thus, the low temperatures and microbial activities found early in this run may have been the result of increased anaerobiasis due to poor aeration. The mean moisture levels of the samples were fairly constant (Figure 32), since all of the samples were taken near the surface of the compost pile (where moisture tends to accumulate). Mean levels of organics declined slightly over time (Figure 33), while the mean

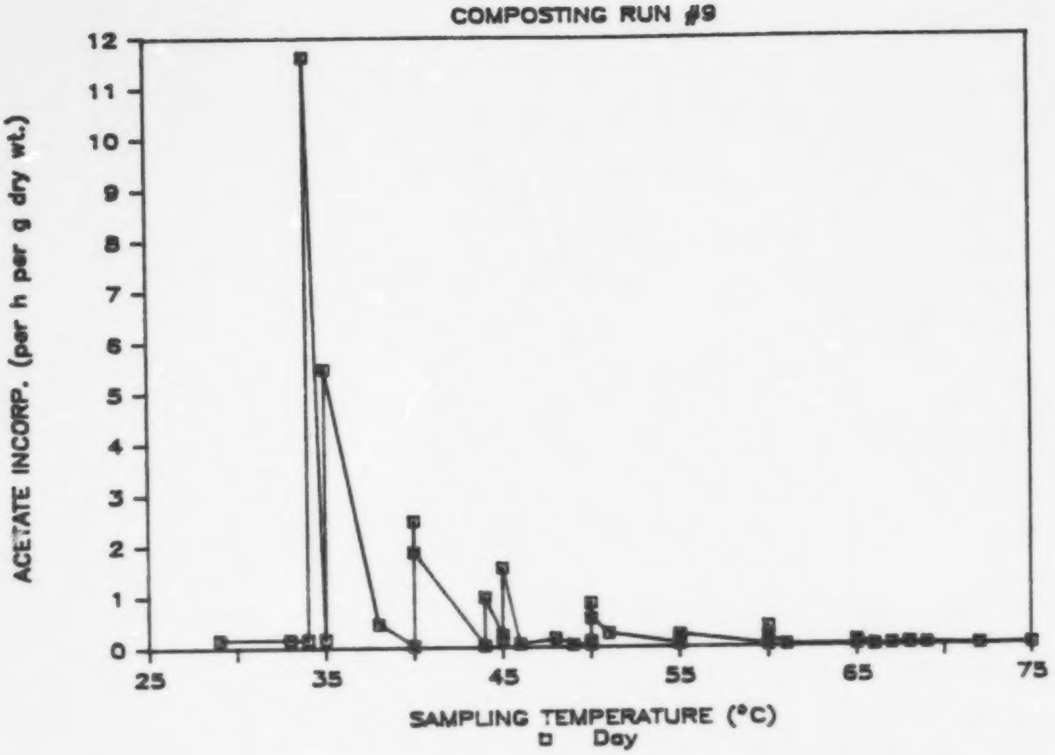

Figure 28. Microbial activity, determined as the rate of [^{14}C]acetate incorporation into lipids per hour per gram of compost, of sewage sludge compost in response to sampling temperature during composting run #9. The values shown are the means of three replicate determinations.

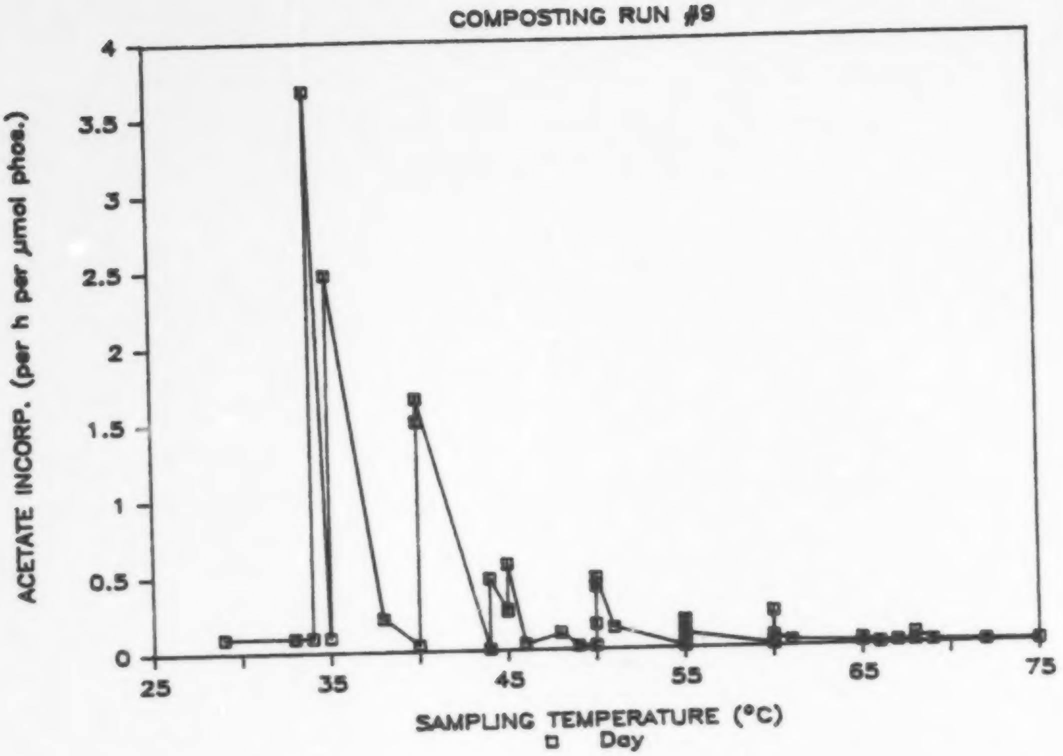

Figure 29. Microbial activity, determined as the rate of [^{14}C]acetate incorporation into lipids per hour per µmol lipid phosphate biomass, of sewage sludge compost in response to sampling temperature during composting run #9. The values shown are the means of three replicate determinations.

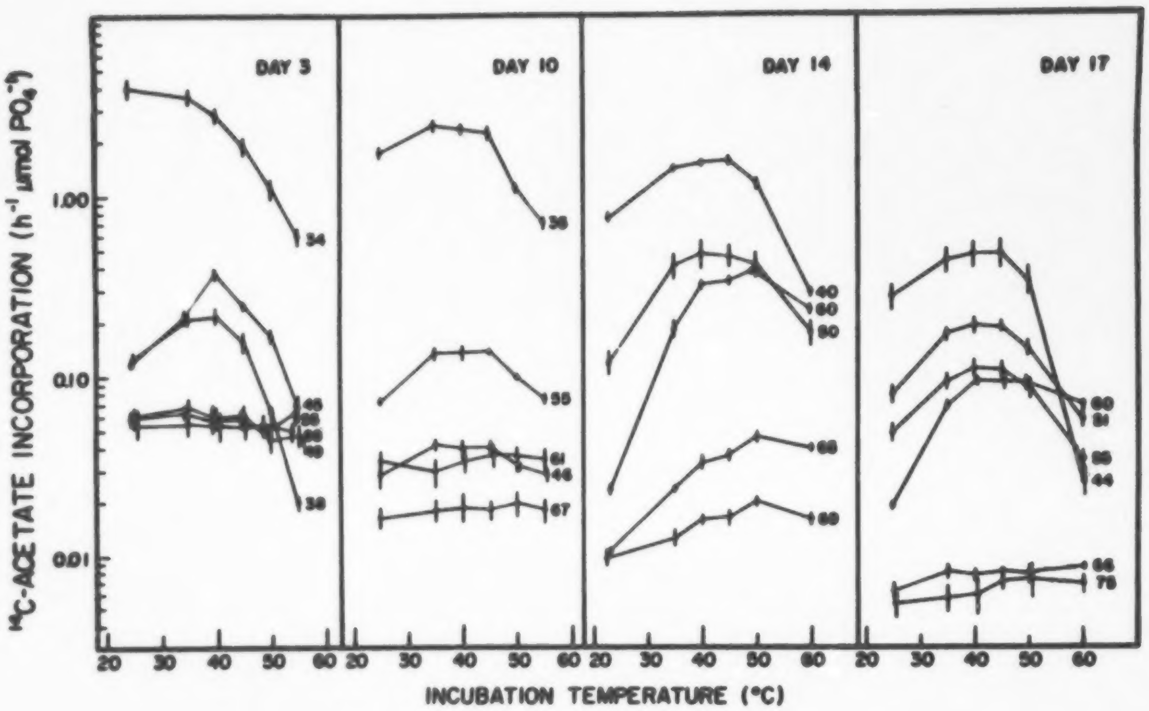

Figure 30. Microbial activity, determined as the rate of incorporation of [^{14}C]acetate into microbial lipids, in composting sewage sludge incubated at various temperatures (± 1.0°C) after 3, 10, 14, and 17 days of composting during run #9. Numbers to the right of each line indicate the sampling temperature (± 0.5°C). Values shown are means ± one standard deviation (n=3).

Table 24: Lipid phosphate biomass and microbial activity at the sampling temperature (rate of C-14 labeled acetate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) of sewage sludge compost samples pooled on each sampling day during composting run #9. Mean sampling temperatures, dry weights, pH values, organics, protein contents and lipid phosphate biomasses for the individual samples which were pooled are also given. The mean compost pile temperature was taken from the 80 temperature thermocouples. Values shown for lipid phosphate and microbial activity of the pooled samples are a mean of three replicates.

Composting Run #9

Day	Mean Pile Temp. (°C)	Mean Sample Temp. (°C)	Mean Sample Dry Wt. (%)	Mean Sample pH	Mean Sample Organics (% of dry)	Mean Sample Protein (% of dry)	Mean Sample Lipid Ph. (µmol/g)	Pooled Sample Lipid Ph. (µmol/g)	Pooled Acetate Incorp. (per g)	Pooled Acetate Incorp. (per PO4)
0	-	33	41.00	6.98	62.88	2.734	-	1.759	0.1711	0.0973
3	46	48	47.06	8.24	63.21	3.204	2.761	4.201	0.0861	0.0205
5	48	55	44.60	7.09	62.01	2.496	2.556	1.399	0.0353	0.0252
7	49	53	49.84	7.67	62.91	2.983	0.766	0.757	0.0442	0.0584
10	51	53	46.93	7.81	60.82	3.086	1.650	1.299	0.0443	0.0341
12	68	60	48.04	8.24	62.96	2.774	1.269	1.400	0.0207	0.0147
14	59	57	43.78	8.68	61.42	3.132	2.053	2.686	0.1251	0.0466
17	63	58	47.69	7.78	62.15	3.310	2.124	2.483	0.0187	0.0075
19	-	47	43.67	8.57	60.30	3.562	1.918	2.258	0.9067	0.4015

Table 25: Matrix of correlation coefficients (r) for microbiological parameters assayed on pooled compost samples (Table 24) during composting run #9.

Correlation Matrix: Run #9 ++

	Pooled Lipid Ph.	Acetate Incorp./PO4	Acetate Incorp./g	Time
Temperature	-0.1582	-0.7033	-0.6715	0.8964 ###
Time	-0.1498	-0.5821	-0.6222	
Acetate/g	0.2647	0.5825 ###		
Acetate/PO4 +	-0.5906			

+ arsine tranformed for ratio correlations with lipid phos.
++ excluding day 19 and using a
ln transform for microbial activity measures.
$p < 0.01$
$p < 0.02$
# $p < 0.05$
$n = 8$

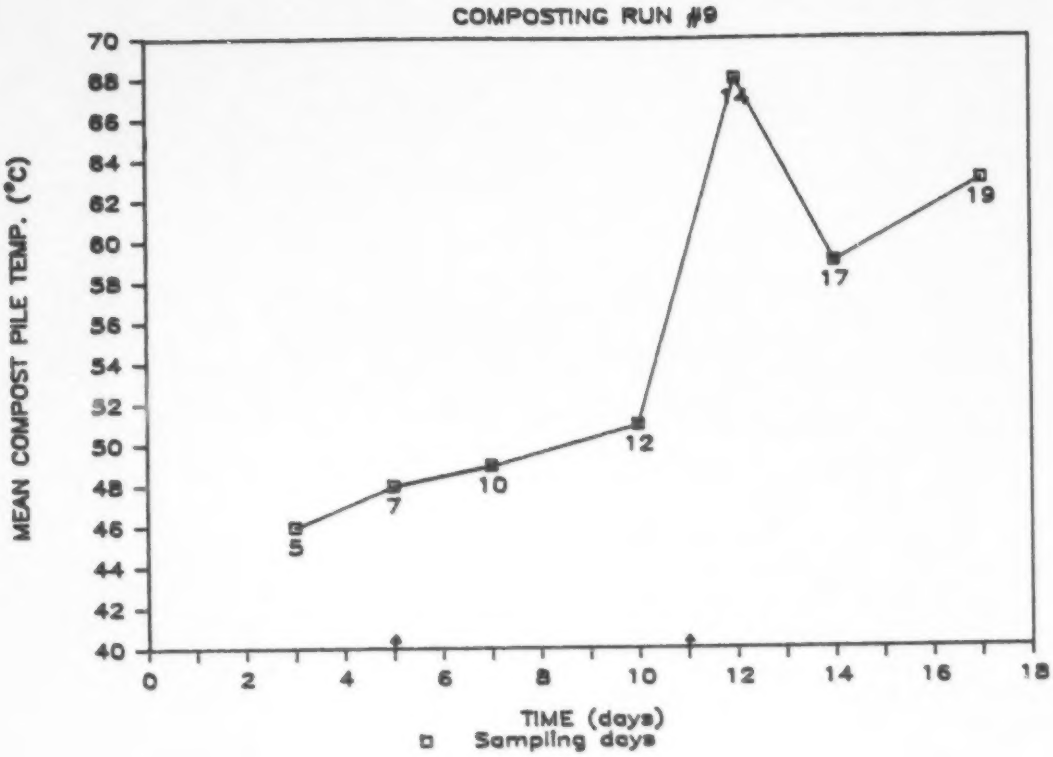

Figure 31. Mean compost pile temperatures in response to time during composting run #9. Sampling days are indicated by the numbers associated with the data points. Temperature readings were taken from thermocouple probes distributed throughout the composting pile (reference 30).

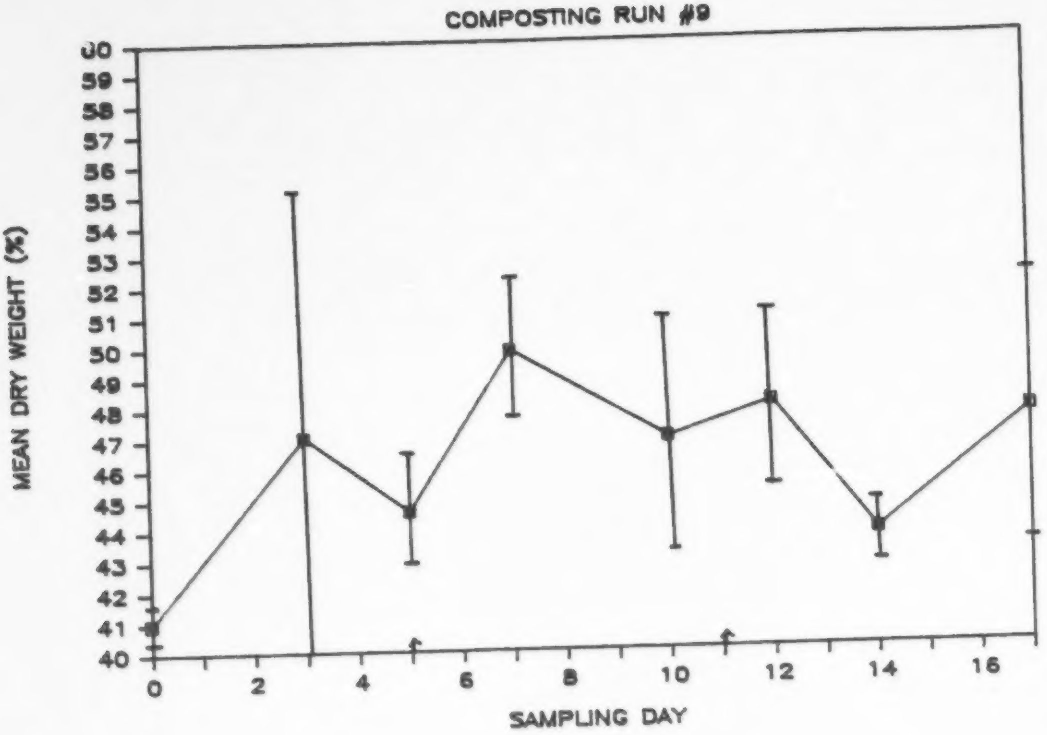

Figure 32. Mean percent dry weight of composting sewage sludge samples taken over time during composting run #9. Values shown are the means of all samples taken on each sampling day (Table 24) ± one standard deviation.

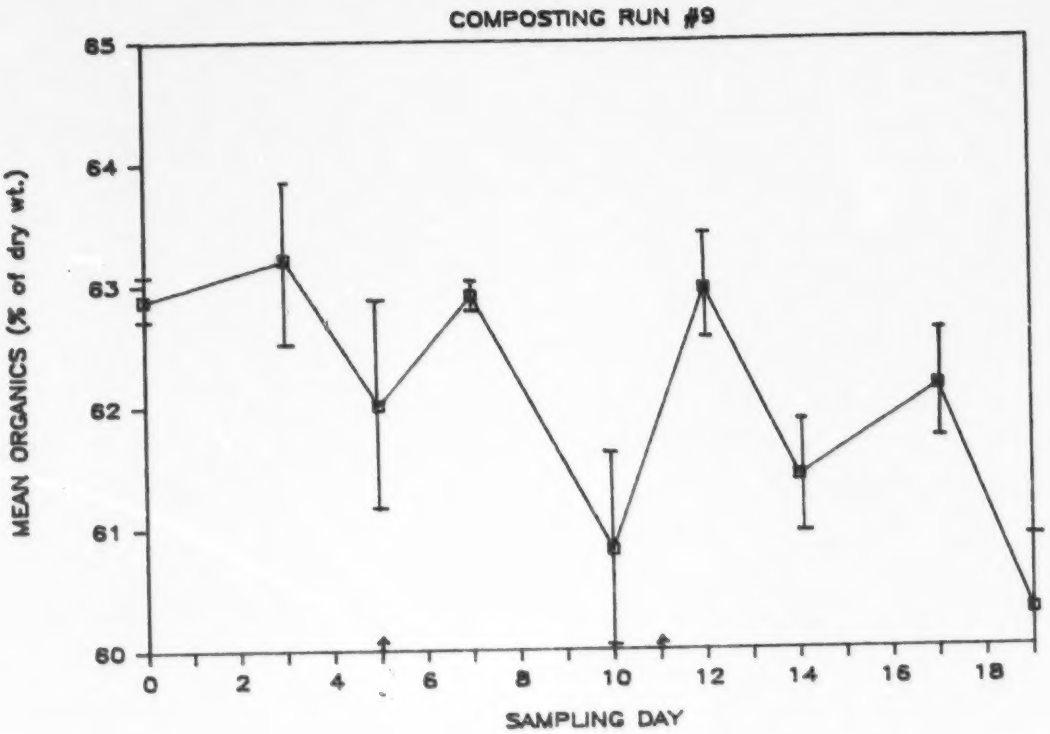

Figure 33. Mean organic content of composting sewage sludge samples taken over time during composting run #9. Values shown are the means of all samples taken on each sampling day (Table 24) ± one standard deviation.

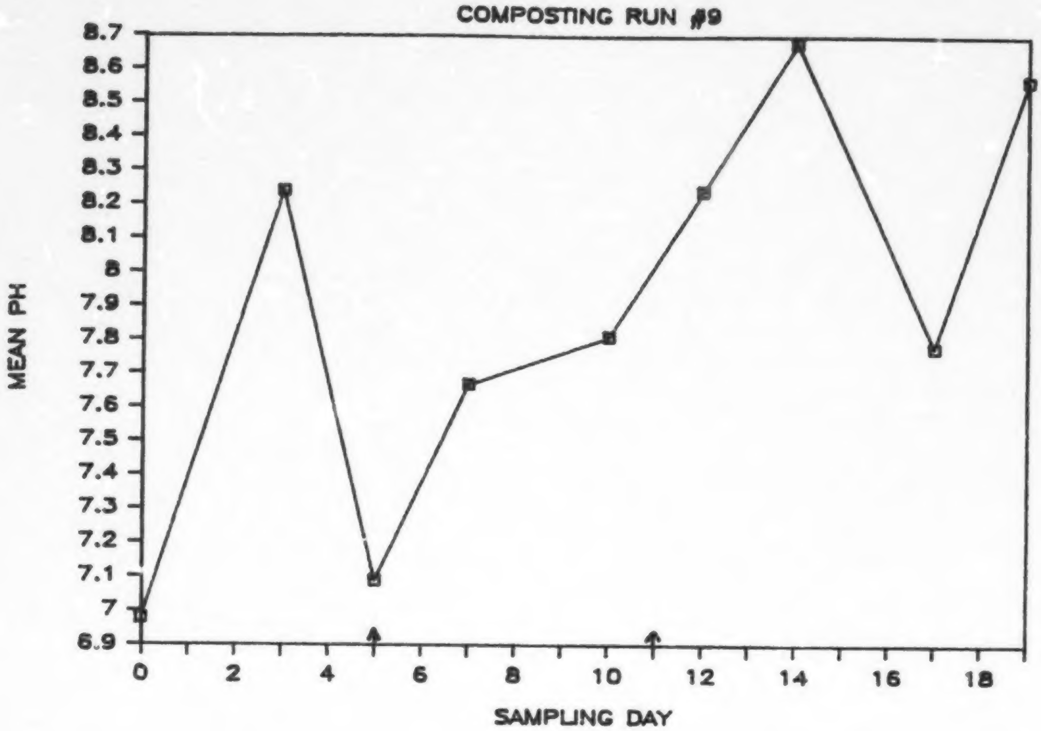

Figure 34. Mean pH values of composting sewage sludge samples taken over time during composting run #9. Values shown are the means of all samples taken on each sampling day (Table 24).

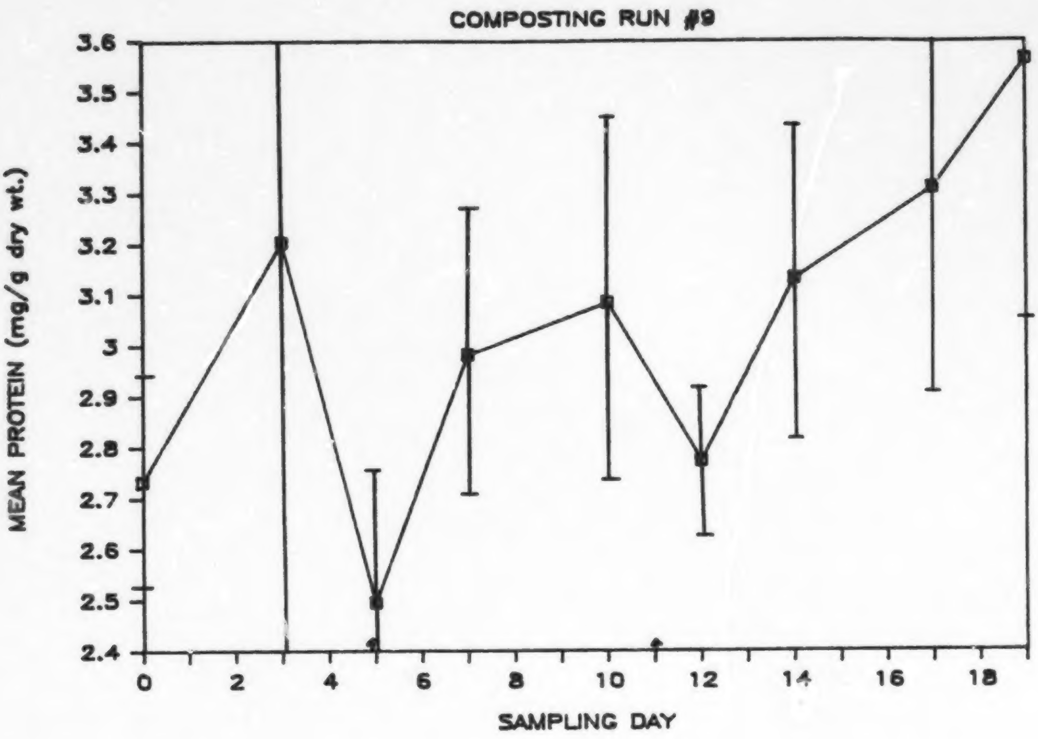

Figure 35.Mean total protein content of composting sewage sludge samples taken over time during composting run #9. Values shown are the means of all samples taken on each sampling day (Table 24) ± one standard deviation.

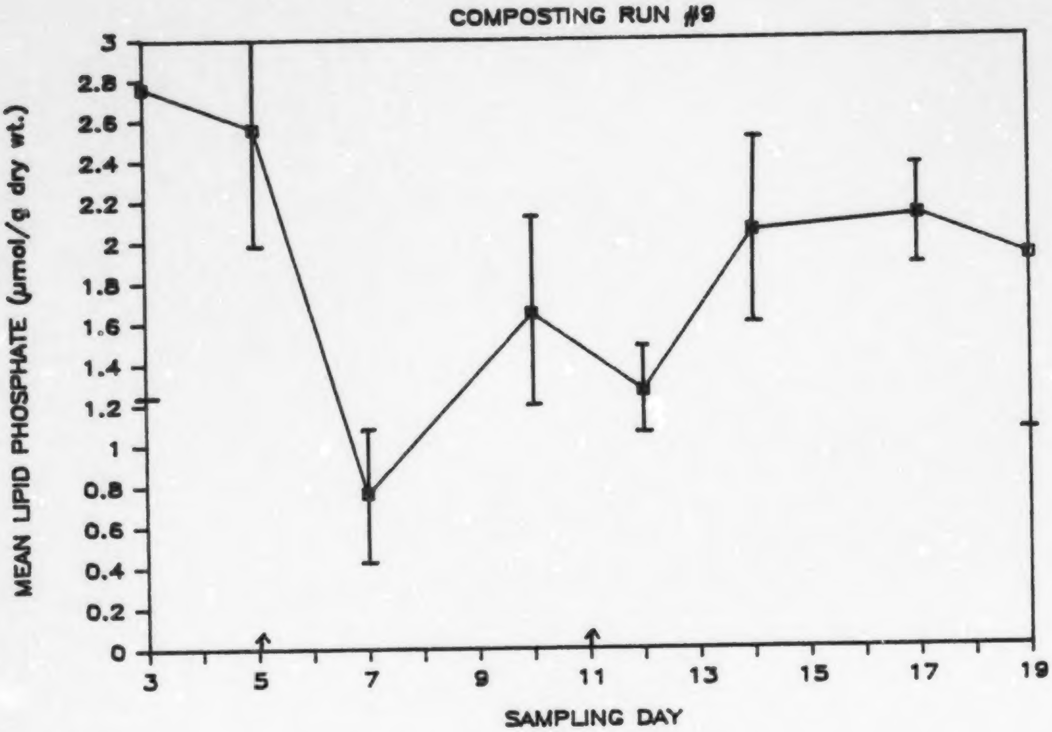

Figure 36. Mean microbial biomass, determined as the lipid phosphate concentration, of composting sewage sludge samples taken over time during composting run #9. Values shown are the means of all samples taken on each sampling day (Table 24).

pH and protein levels rose (Figures 34-35).

The activity curves for the pooled samples (incubated over a range of temperatures) are shown in Figure 37 for four of the sampling days. As with the individual samples, it can be seen that the temperature optima tended to shift toward somewhat higher values as the composting run progressed, but they did not reach the extremely thermophilic range (temperature optima did not exceed 55 to 60°C) in any case. These findings indicate that the microbial communities were probably adapting to increases in temperature to a certain extent (by metabolic adaptation and/or by community succession to a different species composition), but that extreme thermophiles did not play a major role in decomposition, even at high temperatures.

COMPOSTING RUN #10

During run 10 the composting pile was again divided into two seperate sections (or treatments), a low-temperature side and a high-temperature side, to determine whether different temperature regimes (maintained through different aeration rates) could significantly alter the biomass and activity of the microbiota. Three samples were taken from different temperature areas (where possible) near the surfaces of each pile on each sampling day. Table 26 shows the mean compost pile temperature (from the temperature probes), pH, protein, lipid phosphate biomass, and microbial activity (determined as the rate of acetate incorporation into lipids) for each sample. Lipid phosphate biomass was significantly greater in the samples from the low-temperature pile than in those from the high-temperature pile on day 10 (t-test, $p < 0.05$). Microbial activity (acetate incorporation rates) were also significantly greater in the low-temperature pile on days 10, 13, and 15 (t-test). The pH was significantly greater in the low-temperature pile on day 13 (t-test, $p = 0.005$), and was just above the significance level on days 15 ($p=0.056$) and 10 ($p=0.163$).

The correlation matrix (Table 27) once again reveals that lipid phosphate biomass and microbial activity were significantly inversely correlated with sampling temperature. As can be seen in Figure 38, pH was once again generally higher and less variable in the 40 to 60°C temperature range. The correlation between pH and temperature was once again negative, but not statistically significant. Although the correlation between pH and time appears to be slightly negative, going against our previous trends, the partial correlation between pH and time controling for temperature gives a positive correlation coefficient ($r = 0.2592$, $p = 0.145$, n.s.). As was found in run 9, protein content did not show any particular trend in response to sampling temperature (Figure 39), but it did increase somewhat over time (n.s.). Microbial biomass was fairly variable in the samples taken during this run (Figure 40), but it did decrease significantly with increased sampling

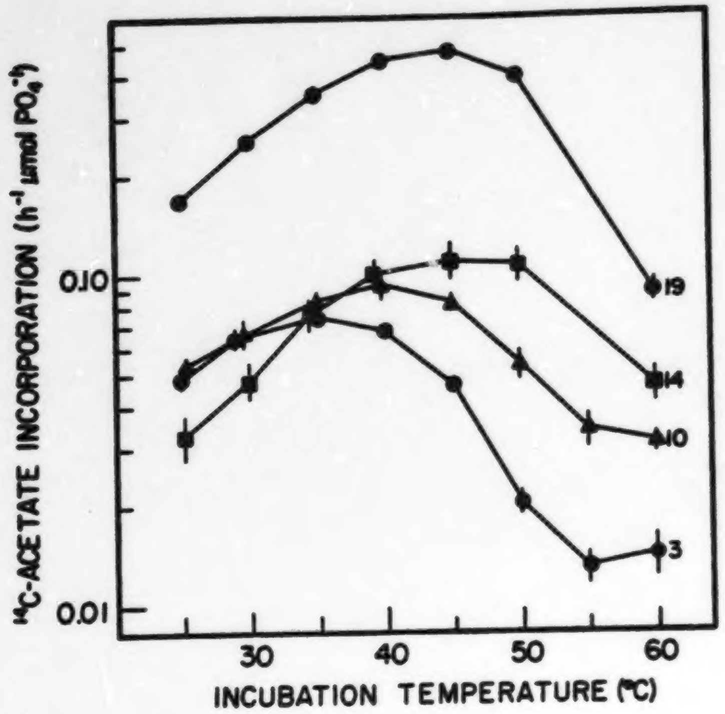

Figure 37. Microbial activities, determined as the rate of [^{14}C]acetate incorporation into lipids, over a range of incubation temperatures (± 1.0°C), for pooled samples of composting sewage sludge taken from a range of sampling temperatures (Table 22) on each particular sampling day (indicated to the right of each line) during composting run #9. Values shown are means ± one standard deviation (n=4).

Table 26: Sampling temperature, pH, protein content, lipid phophate biomass, and microbial activity (rates of C-14 acetate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) for sewage sludge compost samples taken from either the low-temperature or the high-temperature pile during composting run #10. Values shown are the means of three replicates. The mean compost pile temperature was taken from the thermocouple probes.

Composting Run #10 Starting Date: 22 October 1982

Day	Treatment (pile)	Mean Pile Temp.	Sample Temp. (°C)	Sample pH	Total Protein (% of dry)	Lipid Phosphate (µmol/g)	Acetate Incorp. (per g)	Acetate Incorp. (per PO4)
0	low	-	24	6.70	3.290	9.028	0.6109	0.0677
			26	7.52	3.635	4.289	0.9227	0.2151
	high	-	24	6.91	3.290	6.942	0.5543	0.0798
			26	6.79	2.648	6.158	0.3993	0.0648
3	low	44	34	7.77	3.120	5.105	2.2724	0.4451
			40	7.30	3.165	7.133	0.4932	0.0691
			47	7.84	3.000	8.738	2.5243	0.2889
	high	49	47	7.88	3.090	4.036	1.4778	0.3662
			58	7.88	2.687	7.981	1.5492	0.1941
			70	8.05	2.841	7.055	0.2327	0.0330
6	low	51	33	7.65	3.306	10.117	2.6465	0.2616
			41	8.08	3.638	11.046	0.9075	0.0822
			58	8.13	3.119	9.972	1.7408	0.1746
	high	52	51	8.16	3.113	7.995	2.5243	0.3157
			60	8.23	2.943	12.732	1.7801	0.1398
			67	7.88	3.400	10.112	1.7583	0.1739
10	low	-	24	7.83	3.671	11.765	5.2695	0.4479
			50	8.10	3.406	11.908	4.4795	0.3762
			62	7.80	3.710	10.385	0.9159	0.0882
	high	-	50	7.69	3.286	14.493	0.7622	0.0526
			65	6.96	2.989	6.187	0.3182	0.0514
			75	6.60	2.732	4.348	0.2290	0.0527
# 13	low	44	40	8.12	3.158	8.993	4.1796	0.4648
			50	8.10	2.918	10.334	2.8020	0.2712
			60	8.08	3.380	7.408	1.3849	0.1869
	high	68	75	7.98	2.782	7.704	0.1575	0.0204
			80	8.30	2.990	7.602	0.3845	0.0506
			82	7.87	2.995	6.261	0.3263	0.0521
15	low	45	45	7.91	3.009	8.704	9.4170	1.0820
			60	7.88	3.464	7.685	3.5316	0.4596
			65	8.16	3.245	7.669	0.5286	0.0689
	high	69	80	7.57	3.313	6.469	0.5153	0.0797
			80	7.56	3.634	6.345	0.5830	0.0919
			85	7.52	3.802	5.515	0.3254	0.0590

Compost pile was turned on day 10.

Table 27: Matrix of correlation coefficients (r) for the parameters (Table 26) measured during composting run #10. Microbial activity (rates of C-14 acetate incorporation into lipids per gram dry wt. of compost or per µmol lipid phosphate biomass) were assayed at the sampling temperature.

Correlation Matrix: Run #10 +

	Lipid Phosphate	Acetate Incorp./PO4	Acetate Incorp./g	C:N Ratio	Total Carbon	Total Nitrogen	Total Protein	pH	Treatment (pile)	Time
Temperature	-0.4103 *	-0.5987 ***	-0.6849 ***	0.2493	0.1875	0.0318	0.0484	-0.1590	0.6637 ***	0.4572 **
Time	-0.0623	0.0078	-0.0561	0.1794	0.1141	-0.0072	0.3406	-0.0059	0.0000	
Treatment ++	-0.3813 *	-0.5662 ***	-0.5624 ***	0.2657	0.2468	0.0077	-0.3736 *	-0.1658		
pH	0.4225 **	0.2311	0.3403	0.0954	-0.1478	-0.1840	-0.0167			
Protein	0.2348	0.0380	0.1461	0.2182	-0.0922	-0.2311				
Nitrogen	-0.1452	0.0430	-0.0106	-0.3489	0.8078 ***					
Carbon	-0.2664	0.0165	-0.0659	0.2666						
C:N Ratio	-0.1655	-0.0425	-0.0837							
Acetate/g	0.3853 *	0.9311 ***								
Acetate/PO4 +++	0.0954									

+ excluding day 0, and using a ln transform for microbial activity measures

++ Spearman's nonparametric rank correlation coefficient

+++ arcsine transformed for ratio correlations with lipid phosphate

*** $p < 0.01$

** $p < 0.02$

* $p < 0.05$

$n = 30$

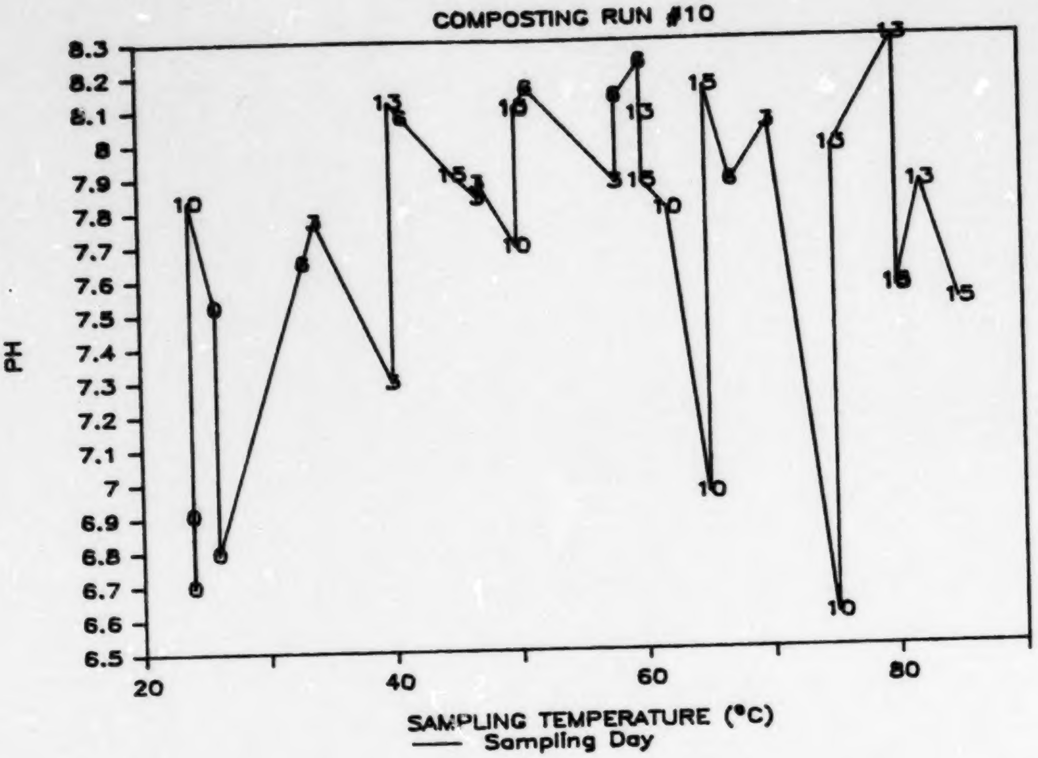

Figure 38. The pH values of sewage sludge compost in response to sampling temperature during composting run #10. The sampling days are indicated by the numbers associated with each data point. The pH values shown are the mean of three replicate determinations.

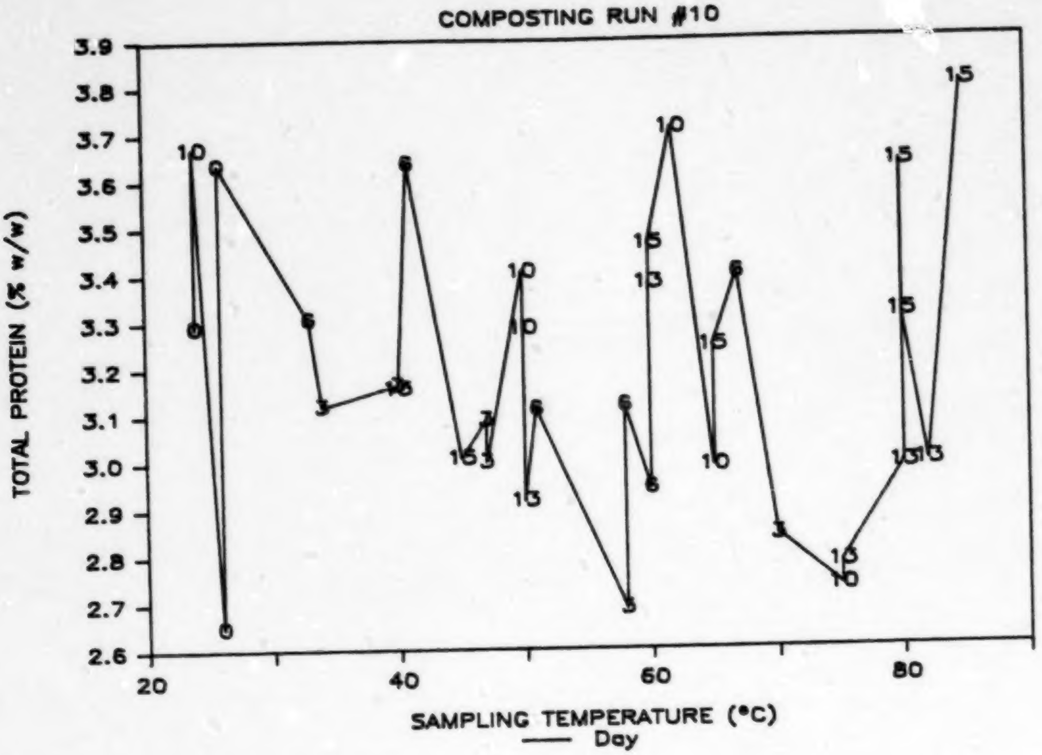

Figure 39. Total protein content of sewage sludge compost in response to sampling temperature during composting run #10. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

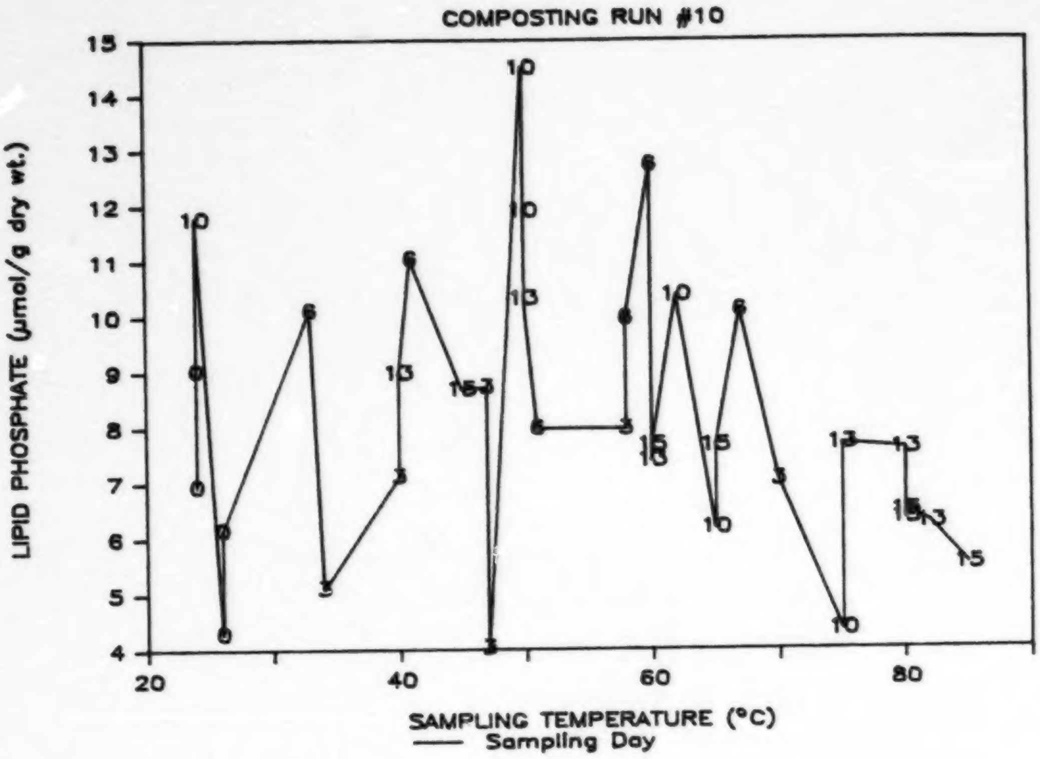

Figure 40. Microbial biomass, determined as the lipid phosphate concentration, of sewage sludge compost in response to sampling temperature during composting run #10. The sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

temperature (Table 27). Microbial activity was maximal between 40 and 50°C (Figure 41-42), and was very low above 60°C.

The same activity data is shown in Figures 43-44 with the data points labled according to treatments (piles). From this representation one can see that, even when samples came from about the same sampling temperature, the activity of the samples from the low-temperature pile were higher than the actvitiy of those from the high-temperature pile. This could be a result of the randomization of the compost in the temperature gradients during turning, such that, even though a sample may currently be in a relatively cool area, it may have previously been very hot. Hence, since in the high-temperature pile the overall temperatures were much higher than in the low-temperature pile, there was a much greater chance that any particular sample may have experienced very high temperatures at one time or another during the run, leading to a decreased microbial biomass and activtiy.

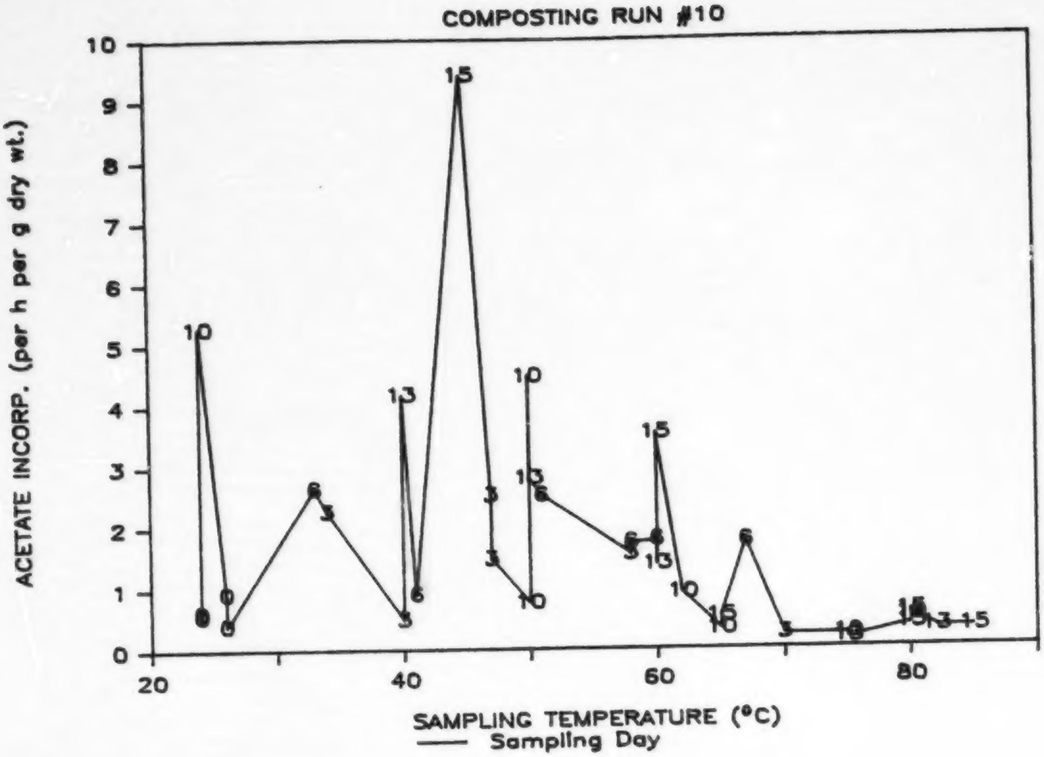

Figure 41. Microbial activity, determined as the rate of [^{14}C]acetate incorporation into lipids per hour per gram of compost, of sewage sludge compost in response to sampling temperature during composting run #10. Sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

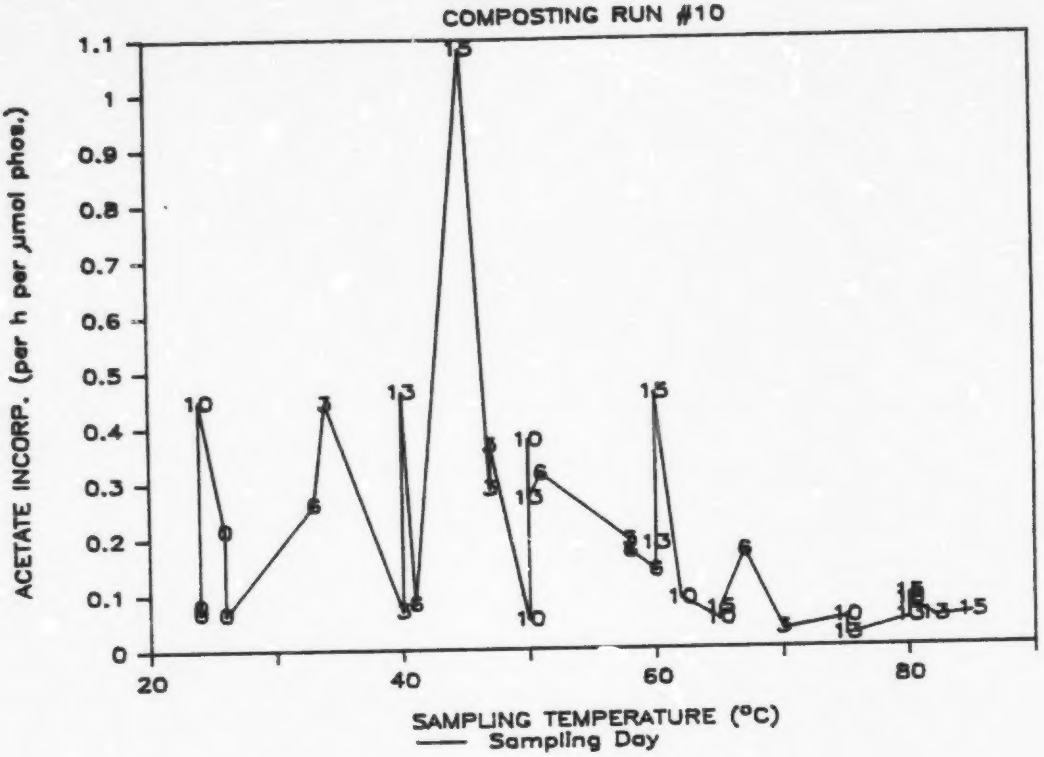

Figure 42. Microbial activity, determined as the rate of $[^{14}C]$acetate incorporation into lipids per hour per µmol lipid phosphate biomass, of sewage sludge compost in response to sampling temperature during composting run #10. Sampling days are indicated by the numbers associated with each data point. The values shown are the means of three replicate determinations.

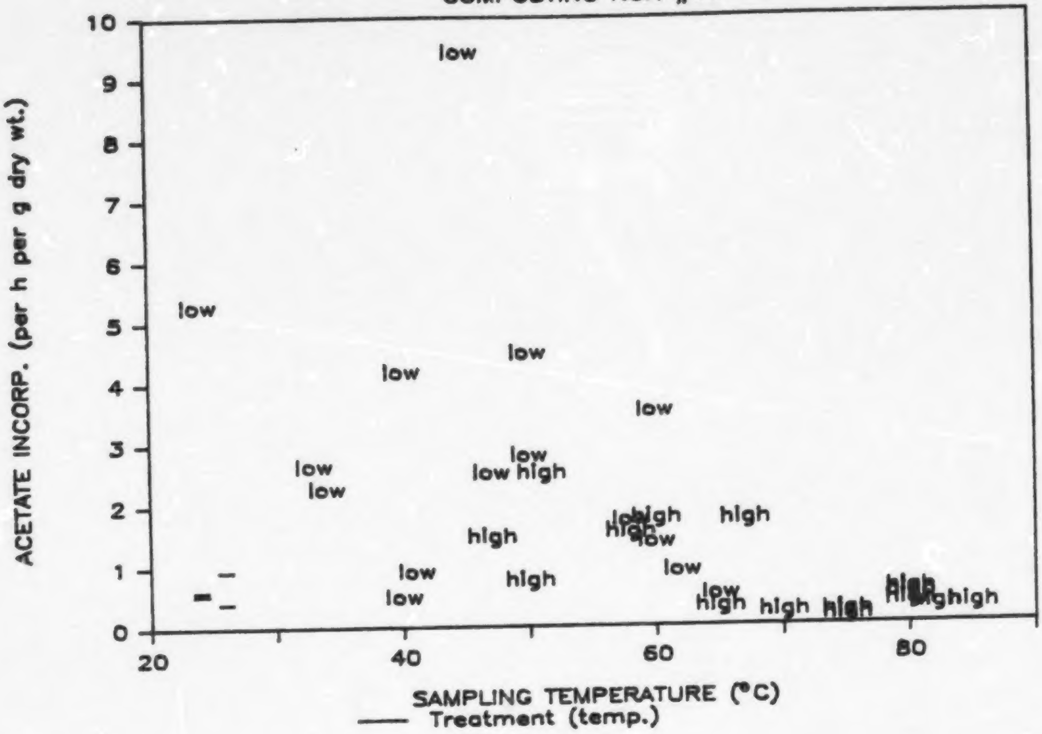

Figure 43. Microbial activity, determined as the rate of [^{14}C]acetate incorporation into lipids per hour per gram of compost, of sewage sludge compost in response to sampling temperature during composting run #10. Treatments (low-temperature or high-temperature composting pile) are indicated by the words associated with each data point. The values shown are the means of three replicate determinations.

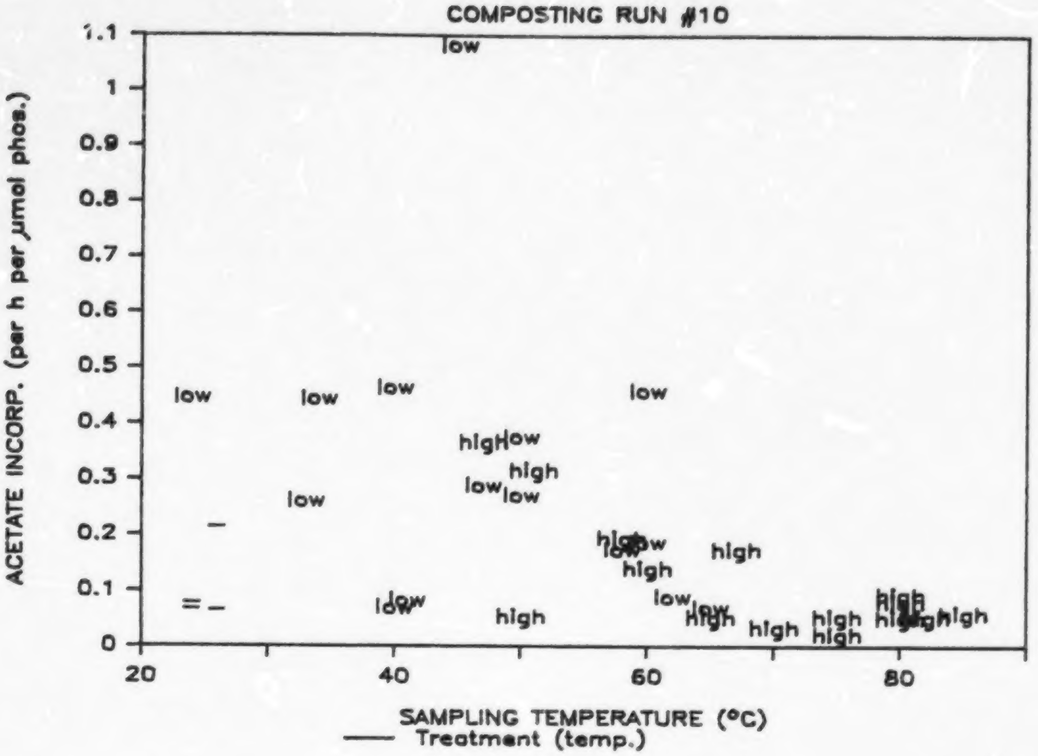

Figure 44. Microbial activity, determined as the rate of [^{14}C]acetate incorporation into lipids per hour per µmol of lipid phosphate, of sewage sludge compost in response to sampling temperature during composting run #10. Treatments (low-temperature or high-temperature composting pile) are indicated by the words associated with each data point. The values shown are the means of three replicate determinations.

SECTION 5

DISCUSSION

PHYSICAL PARAMETERS

Depth

During composting runs 1 through 6, samples were taken at various depths from the upper surface of the composting piles. Depth was found to correlate positively ($p < 0.05$) with % dry weight in the samples taken from 15 and 80 cm depths during composting run 6 (Table 11). The positive correlations between depth and dry weight found for runs 1 and 2 were just below the level of statistical significance. Although the numbers of depths sampled were very limited, these results confirmed our qualitative observations that the uppermost layers of the composting piles tended to remain wet throughout the composting process, while the lower levels tended to dry out more.

Hoitink and Kuter (30) also found significant differences in % dry weight with depth during their more extensive vertical sampling in the preliminary composting trials. They found that the top layers were significantly wetter, but little or no detectible differences in % volatile solids were found with depth. They did not find significant differences in % dry weight or % volatile solids across the horizontal horizons of the piles.

For these reasons, we decided to sample only from the top 5 to 15 cm of the composting piles during runs 7 through 10 such that the effects of temperature on the microbiota could be studied relatively independently of the % dry weight. No other parameters measured besides dry weight were found to correlate with depth.

Moisture Content

Besides the correlations with depth discussed above, the % dry weight of the compost samples also correlated with time during runs 1, 6 and 7. This indicates that there was usually some drying of the material over time, as is desired for this process. However, due to the fact that most of our samples were taken near the surface of the composting piles, where the least amount of drying usually takes place (30), this was not always apparent.

The % dry weight sometimes correlated positively with temperature (run 7), and sometimes correlated negatively with temperature (runs 4 and 8). No consistant pattern was seen in this regard, again possibly due to the sampling locations used. However, the vertical increase in dry weight found by Hoitink and Kuter (30), along with the vertical decrease in temperature (30), would be consistant with the negative correlations.

The generally agreed upon optimum moisture range for composting is 50-60% (40-50% dry weight; 29), or 50-70% (30-50% dry weight; 28) with forced aeration. The % dry weight in our samples ranged from 40 to 69%. Most of the dryer samples came from composting run #4, and the vast majority of the samples analyzed fell within the latter optimum moisture range. Therefore, it would not be expected that moisture limitations would have played much of a role in determining the microbial biomass and activity of these compost samples.

Temperature

As would be expected, the sampling temperatures generally correlated with time during composting (runs 3, 7, 8, 9, and 10). During run 9 the mean sampling temperature on each sampling day corresponded closely to the mean temperature of the composting pile (Table 25), even though all of the samples were taken along the temperature gradients at the surface of the pile. The correlations between sampling temperature and the other paramters measured will be discussed under those headings. More information about the thermal gradients and daily temperatures in the composting piles as a whole during some of these runs can be found in Hoitink and Kuter (30) and in the Akron Composting Demonstration Project Report (Appendix III of 30).

CHEMICAL PARAMETERS

pH

The pH of the individual samples was measured during runs 6 through 10. It was generally found that the pH incresed significantly over time (runs 6, 7, 8, and 9), usually from an initial pH of about 6.5 or 7.0, to a final pH of about 8.2 to 9.0. Although this correlation was not found to be significant during composting run 10, a partial correlation controlling for temperature improved the correlation coefficient considerably.

Increases in pH during composting have been widely described in the literature (5,27,32,34,38,45), but some investigators (45) have also reported an initial drop in pH, followed by a rise. The increases in pH are generally thought to be the result of the volatilization and microbial decomposition of the organic acids which are originally present, and the release of ammonia by microbes mineralizing organic nitrogen souces (e.g. proteins; 21).

The range of pH over which microbial activity has been reported to be optimal in composting systems is very wide. Some investigators have recommended pH 6.0 to 7.5 (43). However, Jeris and Regan (32) performed experiments under conditions of constant temperature and moisture to determine the optimal pH range for composting microorganisms. Over a range of pH 5.6 to 8.4, and at a contant 59.6°C and 65% moisture in a bench-scale composter, they found more microorganisms and higher microbial activity (by Warburg respirometry) at the higher pH levels, ur tp pH 7.8 where there was a leveling off. Acclimation of the composting organisms to the different pH levels prior to experimentation did not affect the results. In a seperate experiment, they found that higher activity could be obtained at low pH (6.0) if the temperatures were lower (46°C), presumably due to the ability of filamentous fungi, which prefer lower pH levels, to grow at these lower temperatures, but not at 60°C. They also found that composted mixed refuse had little buffering capacity.

Although Schulze (reported in 27) maintained therrmophilic composting in a continuous-feed system at pH 5.6, Wiley (reported in 27) found that the addition of acetic acid to compost to maintain a pH $\leq$ 6.0 prevented the generation of thermophilic temperatures in a batch system. Low initial pH also produced a slower initial temperature rise, with temperatures <45°C until pH 7.0 was exceeded, whereupon a rapid increase in temperature to the 60-70°C range occured. It appears that good microbial activity can be found in composts from pH 7.0 to in excess of 8.0. In most of the compost samples for which the pH was determined during this study, the pH was found to exceed pH 7.0 by no later than the third or fourth day of composting, with most of the exceptions being the samples from high (>55°C) sampling temperatures.

Although samples were primarily taken near the surface of the composting piles during the experiments reported here, it is apparent that the pH incresed rapidly after the beginning of each composting run. No evidence for an initial decrease in pH was found. In contrast to the findings of Wiley, samples with temperatures less than 45°C were sometimes found to have pH values in excess of 7.0 (e.g. run 6, day 4), although the starting pH may have been somewhat higher in this case (about pH 6.6). However, it was found during this study that the increase in pH was much more rapid in samples from lower temperature areas of the compost piles (25 to 50°C) than in samples from high-temperature areas (65 to 80°C). This could be seen particularly clearly before the composting piles were turned and all of the material was somewhat randomized with respect to the temperature gradients in the piles (see Table 14 run 7, day 7; Table 18 run 8, day 5; Table 22 run 9, day 5; Table 26 run 10, days 6 to 10). Figures 11, 19, and 23 show that the maximum pH values were generally found in samples coming from 30 to 60°C. In addition, temperatures between 40 and 60°C generally yielded less variable pH values (Figures 23 and 38). These results seem to indicate that incresed pH may be an indirect indicator of high levels of microbial activity, since these temperature ranges for maximal pH values correspond roughly with those for maximal microbial activity.

Organic Matter

During composting run 9 the % total organic matter (% volatile solids) was determined for each compost sample. Although these samples were all taken near the surface of the compost pile and were not meant to be representative of the pile as a whole, a highly significant decrease in total organics was found over time (Table 23). The actual change in the organic content was slight in these samples, however, going from a mean of 62.88% on day 0 to a mean of 60.30% on day 19. The organic content did not correlate significantly with any of the other parameters measured during this run.

Protein

The concentration of total proteins was determined for the compost samples from runs 9 and 10 using the Lowry method. A steady increase in the protein content of the compost occurred throughout these composting runs (Tables 22, 23 and 26; Figure 35). The two days on which the protein content appears to drop during run 9 (Figure 35) are the days during or soon after the turning of the pile. The increase in protein content over time was highly significant during run 9, but fell just beyond the level of significance during run 10. The increase in protein seemed to be somewhat slower at the higher temperatures. Although the initial protein content appeared to be slightly lower in run 9 than in run 10, the samples contained about the same amount of protein at the end of each run (about 3.5% w/w).

The increases in protein content may be primarily a result of increased micribial biomass. However, the protein measurements correlated only slightly with the lipid phosphate biomass measurements from the same samples (r=0.1316 in run 9; r=0.1276 in run 10). This can most probably be attributed to the much higher turnover rate of phospholipids, which makes them a better indicator of viable biomass only. Protein measurements may include a larger proportion of dead biomass, since proteins do not turn over as rapidly, and thus it is possible that the protein levels continued to increase throughout the composting runs as more biomass (dead and live) accumulated. This line of reasoning also accounts for the finding that lipid phosphate correlated much better (negatively) with temperature than did protein measurements. The temperature gradients in the composting piles are not constant, but fluctuate somewhat, and the mixing of the material during turning rearranges the compost even further with respect to these gradients. Therefore, a more slowly decomposed constituent of biomass, such as protein, cannot be expected to reflect the effects of temperature on the microbiota in such a fluctuating system as accurately as a constituent which is more rapidly broken dwn, such as phospholipid.

Waksman (60) also found that protein increased in composting horse manure. In small batches of compost kept a constant temperatures of 28, 50, 65, and 75°C, the most rapid increase in protein occurred at 65°C during the first 9 days, but the 50°C sample was not much slower. Thereafter, the most rapid increase in protein occurred at 50°C up until the end of the experiment at 47 days. Very little increase was seen at 75°C, compared with all of the other samples. These results confirm our assumptions, and correspond well with our temperature optima for microbial activity. Waksman (60) also noted that ammonia accumulated over the first few days of composting, most rapidly where decomposition was most active, and was then quickly assimilated into protein by the microbiota. The ammonia remained unassimilated for the longest period of time at 75°C.

Lipid Phosphate

Lipid phosphate was measured and used as an indicator of microbial biomass in all 10 composting runs. Lipid phosphate levels were generally very high initially, and declined somewhat as the temperatures rose. However, in the samples taken near the surface during composting runs 9 and 10, lipid phosphate levels initially increased somewhat over time, and tended to be lower at the higher temperatures than at the lower temperatures on the same sampling day. Correlation coefficients between lipid phosphate and time were always negative, but were statistically significant only for runs 3 and 4. The high initial values, followed by a decline in many cases, could indicate that either a high initial standing crop of biomass was present from the sewage sludge material (or cow manure in runs 3 and 4), or that the initial rates of phospholipid turnover were low due to a relatively inactive microbial community. The former is a more plausible explanation, since the initial rates of microbial activity were also found to be relatively high.

Correlations between lipid phosphate and sampling temperature were also negative for each composting run, and were significantly so in runs 3, 6, 7, and 10. Three of these runs (runs 3, 7, and 10) were three of the four runs in which high- and low-temperature piles werecomposted seperately. In each of the four composting runs in which the seperate temperature (aeration) treatments were used (runs 3, 4, 7, and 10), the daily mean lipid phosphate values were higher in our samples from the low-temperature pile than in the samples from the high-temperature pile. During run 10, in which the lipid phosphate content was determined for each individual sample rather than for a pooled sample, the difference in lipid phosphate between the two piles increased in significance on each sampling day, up until the piles were turned (t-test: day 3, $p=0.876$; day 6, $p=0.114$; day 10, $p=0.047$; pile was turned on day 10; day 13, $p=0.831$; day 15, $p=0.867$). During run 3 and 4, the difference was not significant, but was just beyond the 0.05 level for a one-tailed t-test due to the rather small sample sizes. These results indicate that the temperature regimes characterized by these different treatments had an effect on the viable microbial biomass present during composting.

Waksman (60) measured biomass by doing plate counts and by counting the types of organisms attatched to microscope slides which had been incubated in composting horse manure. At constant temperatures of 50, 65 and 75°C, he also saw a decline in bacterial biomass over time, although at 50°C fungi and actinomycetes increased over time. Biomass at 75°C decreased from an initial 1.6×10^9 per gram to 3.5×10^8 bacteria (and no other forms) per gram within 8 days. At 50°C, 1×10^9 bacteria and 1×10^9 actinomycetes were present after 8 days, while at 65°C bacteria fell to 1×10^8 by day 2, and fungi were absent. Lehtokari et al (34) found that ATP biomass in a bench-scale composting sewage sludge-bark and wood chip mixture rose with increasing temperature up to about 55°C, and then sharply dropped off as the temperature increased further to about 60°C. As the temperatures began to fall again, the ATP concentrations recovered somewhat.

Lipid phosphate biomass did not correlate with the moisture content, depth, pH (except for run 10), organic matter, or total protein content of the compost samples taken during this study. Lipid phosphate did, however, correlate well with microbial activity during all the composting runs in which microbial activity was determined except for run 7. Thus, it appears that the conditions which favor microbial activity (decomposition) in composting materials also favor microbial growth, and vice versa. During run 7, the correlation coefficients for activity and lipid phosphate were all positive, but not significant due to the very small sample size ($n=4$).

MICROBIAL ACTIVITY

Respiration Rates

During composting runs 7 and 8, the rates of microbial mineralization of [^{14}C]glutamate to $^{14}CO_2$ were measured using radiorespirometry. Mineralization rates log transformed) appeared to vary inversely with sampling and incubation temperatures, as did microbial substrate incorporation rates. Statistcally controling for biomass (lipid phosphate) made the negative correlations between mineralization rate and sampling temperature significant during run 7, as was also true for the incorporation rates.

During composting run 8, the samples taken on each sampling day were pooled, and then assayed for activity at various incubation temperatures. Again, a negative relationship was found consistantly between microbial activity and temperature, but as in run 7 the correlations were not statistically significant until the biomass was statistically controled.

^{14}Mineralization rates correlated well with the rates of [^{14}C]acetate incorporation into microbial lipids, although during composting run 7 the very small sample size precluded statistical significance. Since the two methods of estimating heterotrophic activity yielded similar results, and since the mineralization method was much more time consuming (and was therefore more limiting as to the number of samples which could be analyzed on any particular sampling day), only the incorporation method was used in subsequent composting runs to determine activity.

Incorporation Rates

Rates of [^{14}C]substrate incorporation into microbial lipids were determined during all of the composting runs except for runs 1 and 2. In every case, a decrease in microbial activity occurred as temperatures increased. In most cases where a fairly large number of samples were analyzed the negative correlations between temperature and activity were statistically highly significant (runs 3, 6, 9 and 10) or statistically significant (runs 4 and 8). The same trends were generally seen regardless of whether the activity measurements were expressed as the activity per gram (dry weight) of compost, or whether they were expressed as the activity per umol of lipid phosphate biomass. Thus, not only was the biomass per gram of compost declining with increasing temperature, but the metabolic activity of the "individual" organisms was also declining.

^{14}The incorporation rates of [^{14}C]acetate, [^{14}C]glucose and [^{14}C]glutamate were determined during runs 3, 4, and 6 (Figures 2, 3, 4, 5, 6, 7, 12, 13, and 14). In most cases the responses of the microbial activity as measured by the rates if incorporation of these different substrates were very similar. For this reason, only [^{14}C]acetate was used in subsequent incorporation experiments, since it is a more direct biochemical precursor of fatty acids and lipids.

Of all of the physical and chemical parameters measured during this study, temperature had the most dramatic and consistant effects on microbial activity and biomass in composting municipal sewage sludge or composting cow manure. Microbial activity correlated negatively with time during compoating run 3, but correlated positively with time during run 4. These discrepancies may be due to the fact that time and temperature were also closely correlated during run 3, and that the number of sampling days during these runs were very limited. The early stages of run 3 were not sampled, and sampling was not performed past day 15 during run 4.

During composting runs 9 and 10 pH correlated directly with activity and also with biomass during run 10. This may indicate that microbial activity is responsible for the changes in pH, and that the microbial community can be more active at the higher (neutral to slightly alkaline) pH levels found in composts. These premises were not tested directly, however.

During the composting runs in which seperate sections of the compost piles experienced different aeration rates, and hence different temperatures (runs 3, 4, 7, and 10), microbial activity and biomass were always higher in the low-temperature section than in the high-temperature section on each sampling day. The differences in activity between the two sections were statistically significant on day 7 of run 3 and on days 10, 13, and 15 of run 10 (t-tests). During run 10, the differences between the activities in these samples were greater after the piles were turned, and increased with time. In these experiments it is difficult to strictly distinguish between the effects of temperature and those of aeration, since the temperatures of the composting samples were greater after the piles were turned, and increased with time. In these experiments it is difficult to strictly distinguish between the effects of temperature and those of aeration, since the temperatures of the composting piles were manipulated via aeration. However, any possible oxygen limitations would probably result more directly from the amount of free air space available (packing of the material due to wetness and sizes of clumps; 30) than by these differences in the rates of aeration. No evidence (such as differences in odors) was seen during these composting runs that would indicate that any of the piles became extensively anaerobic. Therefore, it appears that the primary factor affecting the microbial activity and biomass during these composting runs was temperature.

Hoitink and Kuter (30) detected higher levels of carbon dioxide production from the composting piles maintained at lower processing temperatures during the preliminary trials, but as in the study by MacGregor et al (38), measurements of the actual amounts of air forced through the piles were not available, and therefore the actual levels of CO_2 production could not be calculated. However, during the Akron sewage sludge trials II and VII measurements of air flow were made.(30). Trial II corresponds to our composting run 10. During this run the composting pile was divided into a low-temperature (40°C set point) section and a high-temperature (60°C set point) section. A continuous flow of air was regulated by controling the volume of air forced through the piles. Hoitink and Kuter found a very high total weight of carbon dioxide evolved during this trial (30). This "abnormally high" amount of CO_2 evolution may be the result of the abnormally high levels of viable biomass (lipid phosphate) present. The initial lipid phosphate levels measured on day 0 (Table 26) were higher than the levels found in any of the previous samples analyzed, and the levels increased significantly as the run progressed. They also found differences in CO_2 evolution between the low- and high-temperature sections, which also corresponded with the differences they found in the losses of dry solids and volatile solids. Although we did not measure microbial respiration rates directly during this trial (run 10), our microbial incorporation rate data confirm these findings, all of which indicate that the microbiota of the low-temperature section of compost was much more active than that of the high-temperature section. The results obtained from trial VII also confirmed this, with significant differences in CO_2 evolution, volatile solids and dry weight (30).

One should also keep in mind the large amounts of variation in free air space, pH, moisture, nutrients, redox potential, and temperature which typically occur in composting piles due to the solidified and spatially static nature of the system. Such a system makes it difficult to obtain replicability in measurements of physical, chemical and microbial parameters without extensive sampling. The sampling during most of the composting runs reported here was limited to areas near the surfaces of the piles, and the number of samples taken at each sampling temperature on each sampling day were often limited to only 1. In spite of these limitations, the same trends in microbial activity and biomass, particularly in response to temperature, were seen repeatedly. The trends in pH and protein also appear to have been consistant between composting runs, and consistant with the literature. Therefore, we feel confident that the conclusions stated concerning the interactions of these parameters are accurate.

Temperature has been shown to affect rates of microbial decomposition in many systems (22,55). Outside of the composting applications, most of the detailed work on this environmental parameter has been done over comparatively narrow temperature ranges, and usually not under conditions of temperature-driven succession. In the rapid decomposition of organic matter typical of composting, responses of initially diverse (27) microbial communities to rapid temperature increases induced by their own metabolic heat production can be examined. Results of previous studies on the relationships between temperature and microbial activity in composting systems were not in agreement, nor were they comprehensive. Schulze (49) reported that oxygen uptake was directly related to temperature up to 70°C, and several composting systems are apparently operated on this basis (20,27,43,45,46). In contrast, Waksman et al (60), Wiley (64), Jeris and Regan (31), and Finstein's group (26,38,54) report temperature optima for activity in the 55 to 60°C range. Much of these data are, however, difficult to interpret due to variations in composting materials and practices, as well as differences or inadequacies in methods of quantifying microbial biomass and activity. During the present study microbial activities were primarily determined by measuring the rate of incoporation of [^{14}C]acetate into microbial lipids. This method has previously been shown to correlate well with weight loss (24,40), respiration (61), a-D-mannosidase activity (61), and heterotrophic CO_2 fixation (24) in decomposing plant litter, and was shown during these studies to correlate with the rates of incorpoation of [^{14}C]glucose and [^{14}C]glutamate into lipids and with the mineralization rates of [^{14}C]glutamate in composting sewage sludge.

Due to the nature of the incorporation assay, the rates measured may primarily reflect bacterial rather than fungal activity. The role of thermophilic or thermotolerant fungi in the composting process has not yet been established (27). However, the upper temperature limit for fungi is 55 to 60°C (10,12), and it has been found that, in composting systems undergoing temperature increases, the numbers of viable fungi rapidkly decline as the temperatures approach 60°C, and disappear completely at 65°C or less (27,60). Bacteria tend to predominate over fungi throughout the temperature ascent when adequate moisture is present (27). Thus, it is unlikely that thermophilic fungi would contribute significantly to the overall microbial activity, particularly at temperatures greater than 55°C.

It should be cautioned that the estimates of microbial activity made during this study should be viewed as potential activities, rather than actual activities, since the homogenization of the samples during the processing for the assay could disrupt the natural associations between various members of the microbial community, as well as change the immediate chemical environment of the cells.

The shapes of many of the temperature response curves (determined during run 9 when individual compost samples were incubated at several different temperatures; Figures 30 and 37) are typical of those found with purified enzyme systems and pure cultures of bacteria (29,33,57). Jeris and Regan (31) report similar curves for their composting systems, finding that the effect of temperature on oxygen consumption in all of the different materials which they composted could be expressed in the form of

$$y=ax^2+bx+c$$

where y=the rate of composting, x=temperature (degrees Celsius), and a, b, and c are constants. The data presented here fit this form best when a log transform was used, with the exception of those curves which were flat.

The presence of some flat curves (Figure 30), showing little or no significant response to various incubation temperatures, may indicate that the organisms in these samples were so stressed or inactivated that they could not respond. These samples typically had very low levels of activity compared with other samples taken at the same time. This type of inactivation most often occurred in samples taken from very high-temperature (65 to 75°C) areas in the compost pile, although a few such samples came from lower temperatures (49 and 55°C on day 3; 46°C on day 10). The inactivity of these samples could be due to a lack of sufficient oxygen, since they were found early in the run when the aeration levels were abnormally low due to the clogging of the aeration pipes. Odors characteristic of anaerobiasis were detected in some of these samples.

Most of the other temperature response curves look very similar to the Type I curves described by Han (29), which are typically associated with the heat inactivation of enzymes. This is the most common type of nonlinear Arrhenius plot, and is seen when the reaction is modified by a single-equilibrium process (29). The placement of the temperature optimum and the slopes of the lines on either side may be influenced by many different chemical properties of the environment (33). However, the shape of the curve is primarily dependent on the relative rates of two processes having different temperature efficiencies: (i) the rate of the limiting enzyme reaction, and (ii) the equilibrium rate between the active and inactive forms of the enzyme (29,33).

The temperature optima of many of the temperature response curves (Figures 30 and 37) tended to shift toward higher temperatures as the composting run progressed, but they never exceeded about 50°C. This appears to also indicate that, although extreme thermophiles with temperature optima >55°C may have been present, they did not exhibit enough activity (with regard to this assay) at these temperatures to show it. The temperature optima early in the run ranged from 23°C (ambient) to about 40°C. This seems reasonable, since it has been shown that the intestinal microbial communities of mammals are optimally adapted to the body temperature of the host (3).

Allen and Brock (2) found that heterotrophic microcosms seeded with a variety of inocula, including raw sewage, sewage sludge, and hot soils, could be readily optimally adapted to temperatures from 2 to 55°C within a period of 2 weeks. However, growth from 6 0 to 75°C was not established until hotsprings bacteria were added, and even with this treatment no growth was seen at 80 to 85°C. It has also been found that, even when organisms are optimally adapted to a very high temperature, often their efficiency of metabolism and growth are less than those for organisms adapted to lower temperatures (2,10,11,13). Hence, it seems unlikely that rapid rates of growth or decomposition should be expected in composts in which the temperatures exceed 55°C, and even seeding with extremely thermophilic microorganisms would probably not increase the overall efficiency of the process significantly.

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APPENDIX A.

Corresponding dates for composting runs.
Run numbers refer to this study.
Trial numbers refer to previously published reports.*

Run No.	Trial No.	Starting Date**	Ending Date**	Total Days	No. Turns	Sludge Source
	I	12-05-80	02-10-81	68		Columbus
	II	02-11-81	03-03-81	21		Columbus
1	III	05-28-81	06-18-81	22	2	Columbus
2	IV	06-24-81	07-08-81	15	1	Columbus***
3	V	08-13-81	08-24-81	12	0	cow manure
4	VI	08-25-81	09-14-81	21	1	cow manure
5	1	12-17-81	01-20-82	32	1	Akron
6	2	01-22-82	02-18-82	27	2	Akron
-	3	02-19-82	04-05-82	39	4	Akron
7	4	04-05-82	04-22-82	15	2	Akron
8	5	04-23-82	05-13-82	17	2	Akron
-	6	05-14-82	06-08-82	22	2	Akron
9	7	06-11-82	06-30-82	17	2	Akron
-	8	07-02-82	07-15-82	12	1	Akron
-	9	08-06-82	08-25-82	19	2	Akron
-	10	08-27-82	09-20-82	21	2	Akron
-	I	10-01-82	10-15-81	15	1	Akron
10	II	10-23-82	11-08-82	17	1	Akron
	III	11-12-82	12-01-82	20	1	Akron
	IV	12-06-82	01-03-83	29	1	Akron
	V	01-10-83	02-07-83	29	1	Akron
	VI	02-08-83	03-01-83	22	1	Akron
	VII	03-10-83	03-24-83	15	1	Akron

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* reference 30 for Trials I-VI and I-VII;
Appendix III of reference 30 for Trials 1-10.

** Month-Day-Year

*** Sludge was mixed in Columbus.